Proceedings of the 41st Annual Aquatic Toxicity Workshop: September 28 – October 1, 2014, Ottawa, Ontario

Comptes rendus du 41e atelier annuel sur la toxicologie aquatique : du 28 septembre au 1 octobre 2014, Ottawa, Ontario

Editors

C.J. Rickwood, L.N. Taylor, L. Van der Vliet, S.L. MacLeod, A. Dupuis and R. Allen Jarvis

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Rapport technique canadien des sciences halieutiques et aquatiques 3133

Canadian Technical Report of Fisheries and Aquatic Sciences

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Les rapports techniques contiennent des renseignements scientifiques et techniques qui constituent une contribution aux connaissances actuelles, mais qui ne sont pas normalement appropriés pour la publication dans un journal scientifique. Les rapports techniques sont destinés essentiellement à un public international et ils sont distribués à cet échelon. II n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques de Pêches et Océans Canada, c'est-à-dire les sciences halieutiques et aquatiques.

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Les numéros 1 à 456 de cette série ont été publiés à titre de Rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457 à 714 sont parus à titre de Rapports techniques de la Direction générale de la recherche et du développement, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715 à 924 ont été publiés à titre de Rapports techniques du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom actuel de la série a été établi lors de la parution du numéro 925.

Canadian Technical Report of
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2015

PROCEEDINGS OF THE 41ST ANNUAL AQUATIC TOXICITY WORKSHOP: SEPTEMBER 28 - OCTOBER 1, 2014, OTTAWA, ONTARIO

Editors:

C.J. Rickwood[1], L.N. Taylor[2], L. Van der Vliet[2], S.L. MacLeod[2], A. Dupuis[3] and R. Allen Jarvis[4]

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Preface

The 41st annual Aquatic Toxicity Workshop (ATW) was held at the Ottawa Convention Centre in Ottawa, Ontario from September 28 to October 1, 2014. The Workshop included a plenary presentation by Maude Barlow, five concurrent scientific sessions featuring 154 platform presentations, 58 poster presentations and five interactive poster presentations. Total attendance was 316.

This workshop was one of a continuing series of annual workshops in Canada on aquatic and environmental toxicology, covering topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of sediment and water quality criteria. These workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide an annual focus on the principles, current problems and approaches in aquatic toxicology. These workshops are administered by a Board of Directors and organized by local organizing committees. The Proceedings are published with the support of Fisheries and Oceans Canada.

Préface

Le 41ième Atelier annuel sur la toxicité aquatique a eu lieu au Centre des congrès d'Ottawa à Ottawa, Ontario, du 28 septembre au 1 octobre 2014. L'atelier a donné lieu à une communication lors de séance plénière par Maude Barlow, cinq séances scientifiques simultanées qui ont comporté 154 présentations orales, 58 présentations par affiche et cinq présentations interactives par affiche. Trois-cent seize personnes ont assisté à l'atelier.

L'atelier a permis de poursuivre les discussions tenues annuellement au Canada sur la toxicologie aquatique et l'écotoxicologie. Ces ateliers annuels organisés par un comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations publiques et des universités que le domaine intéresse. Ces derniers y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique, mais aussi sur son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements, et la définition de critère pour les sédiments et pour la qualité de l'eau. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publiés avec le soutien du ministre des Pêches et Océans.

Editors' comments

This volume contains papers, abstracts or extended abstracts of all presentations at the workshop. An author index is also included. The papers and abstracts were subject to limited review by the editors but were not subjected to full formal or external review. In most cases, the papers are published as presented and therefore are of various lengths and formats. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are totally those of the speakers and are neither condoned nor rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

The editors would like to thank Dr. Jill Watson for her assistance in preparing these proceedings.

Remarques des éditeurs

Ce compte rendu renferme le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs est aussi inclus. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. La longueur et la forme des communications varient parce que ces dernières sont pour la plupart publiées intégralement. On est prié de communiquer directement avec les auteurs pour faire des remarques sur les travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont celles des conférenciers; elles ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

Les rédacteurs voudraient remercier Jill Watson dans la préparation de ces comptes rendus.

41st Aquatic Toxicity Workshop Organizing Committee / Comité organisateur du 41e atelier de toxicologie aquatique

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PL – Platform presentation
PO – Poster presentation
PLO – Interactive poster presentation

Dr. Richard C. Playle Awards for Outstanding Theses in Aquatic Toxicology

The 2014 winner of the MSc thesis award was Shari Dahmer, University of Waterloo.

Historical trends of polychlorinated dibenzo-p-dioxins and dibenzofurans in white sucker (*Catostomus commersoni*) liver and dated sediment cores from Jackfish Bay, Lake Superior (PL)

Shari Dahmer[1], Roland Hall[2], Kelly Munkittrick[3], Mark McMaster[4] and Mark Servos[2]

[1]Toronto and Region Conservation Authority, [2]University of Waterloo, [3]University of New Brunswick, [4]Environment Canada

Despite improved effluent regulations and an industry shift toward total chlorine-free (TCF) bleaching, previously released polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) produced by pulp mills may remain a concern due to their tendency to persist and bioaccumulate. PCDD/Fs were measured in fish and sediment collected from Jackfish Bay, Lake Superior to document spatial and temporal contamination from bleached kraft pulp mill effluent. PCDD/Fs measured in white sucker (*Catostomus commersoni*) reached peak toxic equivalency (TEQ) in 1991, followed by a rapid decline. Although currently below consumption guidelines and approaching reference TEQ, trace levels of PCDD/Fs persist in exposed fish. These results were consistent with sediment samples, which demonstrated the presence of a unique PCDD/F bleaching pattern in Jackfish Bay. PCDD/F contamination of a dated sediment core from the depositional area illustrated a decline in TEQ, over time, which corresponds to mill process changes. Surface sediment collected throughout Jackfish Bay demonstrated elevated TEQ compared to reference sites, with some locations exceeding sediment quality guidelines. These results suggest that PCDD/Fs associated with particulate organic matter (POM) and dissolved organic matter (DOM), derived from continuous inputs of effluent, historically provided the dominant source of contamination to fish. PCDD/Fs remain elevated in sediment due to historical contamination; however, these are not highly bioavailable and concentrations in white sucker are approaching levels observed in reference fish.

The 2014 winner of the BSc Honours thesis award was Andrew Alexander, University of the Fraser Valley.

Enrichment, isolation, and identification of oil degrading bacteria from the Fraser Valley of British Columbia (PL)

J. Andrew Alexander[1], **Mandeep Saran**[1] **and Stephen Thomas**[1]

[1]University of the Fraser Valley

The petrochemical industry has given humanity immense benefits, allowing us to utilize energy sources and produce a myriad of useful chemicals, including medicines. However, many of these positive attributes have come with an environmental cost. Petrochemical contamination of the environment is extremely pervasive and has a deleterious impact on many ecosystems, sometimes directly affecting public health. In this study, we endeavoured to find and characterize bacterial species that could be useful for bioremediation of environments contaminated with used motor oil. Bacteria were extracted from soil and enriched for oil degrading and metabolizing capability. Following isolation, the bacteria were identified using 16S rRNA whole gene sequencing. Additionally, sequencing of housekeeping genes secA1 and gyrB was attempted to aid in additional confirmation of species identity. This study documents the previously unknown oil degrading capability of several isolated bacteria, including newly characterized *Gordonia*, *Arthrobacter* and *Rhodococcus* species. Future research in this area could lead to the discovery of novel petrochemical metabolism pathways and bacteria suitable for bioremediation. Furthermore, this research contributes knowledge of the microbial bioremediation community in the Fraser Valley that could arise in the event of petrochemical contamination.

Behavioural Analysis in Toxicology Research and as Biological Early Warning Systems (BEWS) for Environmental Monitoring

The interactive poster session was a new feature at ATW 2014 and followed both a platform and poster procedure. Each presenter first gave a brief platform presentation on the project, with a few minutes for questions. At the end of the presentations, the audience was invited to visit the posters for more in-depth discussions. The posters were situated adjacent to the platform presentation area and therefore allowed audience members to access the posters immediately following the platform presentations.

Assessing the utility of behavioural analysis for toxicology (PLO)

Steven Melvin[1] **and Scott Wilson**[1]

[1]CQUniversity Australia

Environmental contaminants are frequently identified at low concentrations in wastewaters and natural waterways around the world, indicating a need for rapid and sensitive tools to facilitate efficient toxicological risk assessment. There is growing interest in behavioural analysis as one option for satisfying this need, but it is not clear how well behavioural techniques actually compare to tried and true toxicological methods like calculations of acutely lethal concentrations, or the assessment of developmental and reproductive effects. To this end, we performed a meta-analysis comparing the relative sensitivities and average durations of behavioural studies to those assessing acute lethality, developmental and reproductive endpoints. Results indicate that research assessing behavioural endpoints is often significantly faster and comparatively more sensitive than traditional developmental or reproductive studies, for a wide range of environmental contaminants. We also found statistical power achieved through analysis of behavioural endpoints to be higher compared to studies assessing developmental or reproductive endpoints, even though effect sizes are often smaller. As such, there is incentive for research aimed at developing and optimizing techniques for behavioural analysis, since this could contribute significantly to the tools available for ecotoxicology and environmental monitoring. However, it is important to note that for behavioural methodologies to offer truly meaningful results and interpretations, research must be directed at understanding how changes to discrete behavioural indicators relate to endpoints of broader ecological concern, such as survival and organism health and fitness.

Behavioural effects of acute exposure to fluoxetine and wastewater effluent in the invasive round goby (PLO)

Erin McCallum[1] **and Sigal Balshine**[1]

[1]McMaster University

Behaviour is increasingly being used as a toxicological tool to assess sensitive perturbations caused by contaminants. Behaviour can be especially informative when the contaminants of concern are designed to modulate human behaviours and physiology, such as pharmaceuticals. Given the increasing occurrence of pharmaceuticals in the environment, there is concern over whether these compounds may be affecting sensitive behaviours important for fitness in organisms exposed in the wild. In two separate experiments, we exposed male and female round goby to environmentally relevant concentrations of the antidepressant fluoxetine (0 $\mu g \cdot L^{-1}$, 1 $\mu g \cdot L^{-1}$, 40 $\mu g \cdot L^{-1}$), and treated wastewater effluent (0%, 10%, 50%). After 72 hours, we assessed the behavioural effects of these exposures using three assays targeting a range of behaviours: dispersal and exploration, aggressive contests, and social affiliation. We found that exposure to fluoxetine resulted in decreased aggression in both the contest and social affiliation assays, but did not impact dispersal and exploration. In contrast, we found reduced activity after exposure to wastewater effluent, but no clear trends in aggression and social affiliation. Our results will help develop behaviour as a tool for assessing pharmaceuticals' effects in the wild, and emphasize that controlled exposure to single contaminants in the lab may not always accurately reflect effects of the combinations of contaminants that are being experienced by wild populations.

Behavioural effects of coal mine wastewater on an Australian fish species (PLO)

Chantal Lanctôt[1]**, Scott Wilson**[1]**, Larelle Fabbro**[1]**, Frederic Leusch**[2] **and Steven Melvin**[1]

[1]Central Queensland University, [2]Griffith University

Coal mining represents a major economic activity, particularly so in Australia, but concerns about potential environmental effects necessitate effective monitoring tools. Substantial volumes of mine wastewater may be discharged into the environment, through both planned and accidental releases. These discharges can be highly saline and often contain high levels of dissolved solids, suspended solids, heavy metals, hydrocarbons and other compounds. Traditional toxicological testing has generally involved assessment of acute toxicity or chronic toxicity with longer-term tests, and while such tests provide useful information, they are poorly suited to ongoing monitoring or rapid assessment following accidental discharge events. We therefore investigated the use of behavioural responses of native Australian fish as a tool for evaluating potential toxicological effects of coal mine wastewater releases on freshwater ecosystems. Empire gudgeons (*Hypseleotris compressa*) were exposed to wastewater from two dams located

at an open-cut coal mine in Central Queensland, Australia. Fish were exposed for two weeks and behaviour was monitored using the Multispecies Freshwater Biomonitor® (LimCo International GmbH). Exposure to 100% coal mine wastewater resulted in less time spent swimming (low frequency activity, 0-0.5Hz) and increased ventilation rate (high frequency activity, 2.5-3.0Hz) compared to controls. These effects were observed within 24 hours of exposure and persisted throughout the exposure. The results indicate the potential for using behavioural endpoints in native fish species as tools for monitoring wastewater discharges. However, future experiments are needed to investigate the sensitivity of these tools for realistic environmental concentrations, and the practicality of applying this technique to *in situ* monitoring during the wet season.

Toxicity assessment of the antimicrobial triclocarban using a sublethal behavioural scoring system for *Daphnia magna* and *Lumbriculus variegatus* (PLO)

Melanie Raby[1] **and Lynda McCarthy**[1]

[1]Ryerson University

Aquatic environments have long been used as disposal sites for domestic and industrial wastes, resulting in increasing chemical contamination, decreased water quality, and concern for ecosystem health and drinking water sources. Municipal wastewater effluent contains innumerable “down-the-drain” contaminants, including the widely found antimicrobial triclocarban. This study developed a simple behavioural scoring system to assess changes to swimming and locomotion behaviour of the aquatic model organisms *Daphnia magna* and *Lumbriculus variegatus*. The system was developed and demonstrated using the positive reference toxicant 4-chlorophenol before being implemented to assess the impact of environmentally-relevant concentrations of triclocarban. Concentrations of 10.0 $\mu g \cdot L^{-1}$ triclocarban showed a possible impact to behaviour for both *D. magna* and *L. variegatus* swimming and locomotion.

The role of chemokines in lateral line development of the frog, *Silurana tropicalis* (PLO)

Natasha Horsman[1] **and Vance Trudeau**[1]

[1]University of Ottawa

Endocrine systems interact with the nervous system in various ways; understanding these interactions can provide insight into the adverse effects of endocrine disrupting chemicals on an organism’s nervous system. To determine the impact of endocrine disrupting chemicals on the nervous system development of the model organism, *Silurana tropicalis*, we first must understand the normal mechanisms driving nervous system development. The lateral line, a neuronal system critical to the survival

and functioning of aquatic vertebrates, can act as an indicator of neuroendocrine disruption because it is on the surface of the skin, where physiological effects such as delays and disruption in migration can be easily observed. The lateral line system also plays a significant role in survival and behaviour. The stromal cell-derived factor 1 chemokine receptor 4 axis is critical in the development of the zebrafish (*Danio rerio*) lateral line and is a pathway susceptible to oestrogenic endocrine disruption. We investigated the effects of Plerixafor, a chemokine receptor 4-specific antagonist, on survival, growth, and development of anuran member *S. tropicalis*. We hypothesized that the stromal cell-derived factor 1 chemokine receptor 4 axis follows zebrafish development and is responsible for the controlled migration of the posterior lateral line neuromast primordial cells in *S. tropicalis*. Thus, the purpose of this study is to determine if exposure to Plerixafor disrupts the migration and deposition of the neuromast organs and to determine an LC_{50} for Plerixafor for amphibians. In order to test the hypothesis, we needed to develop an entirely novel set of protocol to observe and measure changes in the posterior lateral line. Embryos were exposed to 1-100 $mg \cdot L^{-1}$ Plerixafor prior to the onset of lateral line development, corresponding to the Nieuwkoop & Faber stage 22, for a total of six days before sampling and analysis. We have not found a median lethal dose and have concluded that Plerixafor is developmentally toxic at 100 $mg \cdot L^{-1}$. Significant abnormalities of full body edemas and malformed spines were induced by 100 $mg \cdot L^{-1}$ Plerixafor exposures, and changes in swimming behaviours were observed, which suggests that Plerixafor is toxic to larval development. These findings provide a launching pad for future studies into both the developmental pathway in lateral line development and the environmental impact of the anticipated exponential increase in use of chemokine receptor 4-specific antagonists in the treatment of disease pathways.

Advances in Aquatic Toxicology

Exposure-dose relationships in aquatic toxicity: Interaction of test design, modifying factors and dose surrogates (PL)

Lynn McCarty[1], Don Mackay[2] and Jon Arnot[3]

[1]L.S. McCarty Scientific Research & Consulting, [2]Trent University, [3]Arnot Research and Consulting Inc.

Although interest in new testing methods continues unabated, the issue of whether current methods are adequate for the increasingly sophisticated objectives of modern regulatory toxicology does not receive much scrutiny. To this end, the relationship between exposure-based metrics such as LC_{50} and organism-based metrics such as Critical Body Residue (CBR) as indicators of chemical toxicity is evaluated. A simple one-compartment toxicokinetic mass-balance model for water-exposed fish is employed with a series of hypothetical organic chemicals (log K_{OW} 0 to 8). The influence of several toxicity-modifying factors is examined for fixed-duration and steady-state exposures. The results show that standard toxicity tests such as the LC_{50} are strongly influenced by modifying factors such as hydrophobicity, exposure duration, body size, lipid content, metabolic biotransformation and mode of action. Although the nature and degree of influence varies, it can be significant, up to ~1,000 to ~10,000 times between fixed duration and steady-state LC_{50}s. Dominance of the whole-body concentration by the hydrophilic organism phase for low log K_{OW} chemicals and by the non-target hydrophobic organism subphase for high log K_{OW} chemicals can further confound LC_{50}-CBR_{50} dose surrogate relationships. These influences are not addressed in standard aquatic testing. Consequently, standard aquatic toxicity test metrics such as LC_{50}s are not consistent dose surrogates and may be inappropriate for the basic toxicological task of comparing the relative toxicity of chemicals. Dose metrics such as CBR are preferable. However, as they are also dose surrogates, there are still constraints, not the least of which is the lack of toxicokinetic and CBR data collection in standard testing. Changes in testing protocols, including the use of models in advance of empirical testing, are needed to improve efficiency and effectiveness and clarify confounding toxicity-modifying factor influences. This will assist in linking empirical measurements of LC_{50}s and CBRs, two different but related indicators of aquatic toxicity, and thereby improve understanding of the large existing database of aquatic toxicity test results.

Robust study summary (PL)

Kerry Ketcheson[1]

[1]Environment Canada

Environment Canada's Environmental Emergencies Program has developed an in-house spreadsheet for use in risk assessment of chemicals. The spreadsheet, adopted and modified from the Organisation for Economic Co-operation and Development (OECD), serves as a "robust study summary" to assist risk managers in Environmental Emergencies Program to objectively evaluate laboratory experiments for fish toxicity, such as acute toxicity LC_{50} 96-hour studies for Canadian fish species. The robust study analysis is used to validate the LC_{50} 96-hour concentration for recommending aquatic toxicity-based thresholds for the *Environmental Emergency Regulations*. The presentation will provide background information on the *Environmental Emergency Regulations* and a demonstration of the robust study analysis using the spreadsheet.

Biological method development: Why not keep quality in mind? (PL)

Trudy Watson-Leung[1]**, Jordana Van Geest**[2] **and David Poirier**[1]

[1]Ontario Ministry of the Environment and Climate Change, [2]Golder Associates

From 2007 to 2010, the development of an Ontario Ministry of the Environment laboratory method to assess bioaccumulation of contaminants from field-collected sediments in three freshwater organisms was undertaken in partnership with the University of Guelph. From the very beginning the method was developed with standardization in mind. After a very thorough literature review of current practice in bioaccumulation assessments, meticulous thought went into assessing the impact of key variables on data quality (e.g., loading density, organism size, standardizing sediment total organic carbon). In the end, a PhD thesis and "Bioaccumulation of sediment-associated contaminants in freshwater organisms" method were complete. Then came the task of putting this method into practice in a production laboratory for new sediments and new and emerging compounds. The authors will discuss how the process of validating, accrediting and co-ordinating an inter-laboratory assessment of a new method has posed both interesting and frustrating challenges. The authors will also describe how keeping in mind a framework for method development and validation based on International Standards Organization (ISO) 17025 principles can strengthen the value and credibility of any new research/methodology.

Ensuring the quality of aquatic toxicity test results (PL)

Curtis Eickhoff[1]

[1]Maxxam Analytics

To function correctly, industrial and commercial chemical formulations are often made to be poorly soluble in water. For example, inks and dyes, pesticides, pharmaceutical formulations, lubricants, oils, and petrochemical-based products are necessarily insoluble in water and this desirable property makes them effective. However, this same property makes aquatic toxicity testing challenging and can result in erroneous test endpoints if steps are not taken to analyze these test items properly. Regulatory authorities and risk assessors require reliability and accuracy in toxicity testing data in order to make decisions about the environmental safety of these products. Testing poorly soluble test items is not as simple as adding them to water in a fish tank and giving the solution a stir prior to adding fish. Strategies for dealing with poorly soluble test items to ensure reliable results include: (a) testing to the limit of water solubility; (b) preparing water accommodated or soluble fractions (WAF, WSF); (c) using generator columns or other methods for introducing the test item to the test medium; and, (d) using confirmatory chemical analysis for determining measured concentrations of the soluble test item in test solutions (important for endpoint determination and ensuring data quality). Interpretation of toxicity test results for poorly soluble test items also requires special attention. For example, one might ask, “Were biological or toxicity effects caused by the chemical acting on the test organism after absorption or externally through physical processes?” This presentation will discuss many of these considerations that must be addressed by chemical test sponsors, laboratories conducting toxicity tests, regulators and risk assessors in order to ensure that toxicity analysis accurately reflects the toxic potential of poorly soluble test items.

Processing large data sets in a large country: How to characterize the toxicity of hazardous noxious substances (HNS) in Canadian waters in support of a spill risk assessment (PL)

Jérôme Marty[1], **Steve Potter**[2], **Catherine Tardy Laporte**[1] **and Catherine Lalumière**[1]

[1]WSP Canada, [2]SL Ross Environmental Research

Assessing hazardous noxious substances (HNS) toxicity is challenging because of the diversity of substances considered as HNS, which are associated with different behaviours, potentially causing a wide range of impact when released in the environment. As a consequence, very few risk assessments have been conducted for HNS, particularly on a large geographic scale. This study presents a method developed to characterize the toxicity of five chemical categories moved as bulk substances in Canadian waters: liquefied gas, coke and asphalt products, organic substances, inorganic substances, and vegetable/animal oil. For each category, a hazard index (HI) was computed using

chemical-specific information describing the stability, flammability, compatibility with water, and the toxicity to biota. The HI also included an estimation of the relative volume of each chemical compared to the overall volume of each class in a given geographic sector. Based on this approach, toxicity maps across the country can be produced and applied when evaluating risks of spills at a large scale.

Whole body concentrations of major cation levels in *Daphnia pulicaria* throughout a 24-hour exposure to soft water LC_{50}s for hexavalent chromium, copper and nickel (PL)

John Bailey[1], Martha Celis-Salgado[2], Vincent Evans-Lucy[2] and Jocelyn Anderson[2]

[1]Ontario Ministry of the Environment and Climate Change / Laurentian University, [2]York University

Changes in whole-animal concentrations of major cations can indicate physiological responses to exposure to stress. We determined the LC_{50}s for hexavalent chromium, copper and nickel in a soft water medium (FLAMES medium) for adult *Daphnia pulicaria*. In three separate experiments, we then exposed *D. pulicaria* to the soft water LC_{50} values for the three metals, with replicates and controls. Twenty animals were removed from the treatments and controls following 1, 3, 6, 9, 12 and 24 hours of exposure. Each sample of 20 animals was dried and weighed, acid-digested and analysed for magnesium, potassium, calcium and sodium. The changes in the levels of these cations in animals exposed to the metals over the 24-hour period were compared to the controls and we evaluated what physiological processes in *Daphnia pulicaria* may be affected by these exposures. This study provided insight into the effects of hexavalent chromium, copper and nickel on these animals in soft-water aquatic systems, information that will be used to establish exposure times and assist with interpretation of mass spectrometry results in future metabolomics studies.

Toxicity of three lanthanides on the green alga, *Chlorella fusca* (PL)

Cédrick Beaubien[1], Sébastien Leguay[1], Peter G.C. Campbell[1] and Claude Fortin[1]

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Rare-earth elements (REEs) are widely used in electronic devices (cell phones, lasers, televisions, etc.). However, not enough ecotoxicological data are available in the literature to perform an ecological risk assessment (ERA) of these elements. Since major mining and resource development activities are occurring in the northern ecosystems of Canada, the need for such ERA-relevant data is becoming urgent. At constant pH and water hardness, metal toxicity is usually proportional to the free metal ion concentration. However, the links between metal speciation and bioavailability have not yet been explored for the rare-earth elements. In this project, we are exploring the toxic effects of

three lanthanides (lanthanum (La), cerium (Ce) and neodymium (Nd)) on a green unicellular alga as a function of metal speciation. Since lanthanides have high affinity for phosphate (PO_4^{3-}), which is normally used in algal culture media, algal metal exposures need to be done in a phosphate-free medium. For this purpose, we have used the green alga *Chlorella fusca*, which can grow for several days on its P reserves. The modified MHSM-1 medium buffered with 2-(N-morpholino)ethanesulfonic acid (MES) at pH 5.5 is used to grow *C. fusca*. In order to use the algae for metal exposures, they are harvested on a 2-µm polycarbonate membrane and then washed and resuspended in the MHSM-1 medium without phosphate or trace elements. The algae are then added to the exposure flasks at an initial algal density of approximately 15,000 cells per mL. Preliminary chronic exposure experiments (120 hours, pH=5.5) were performed with *C. fusca* at different concentrations of La and Ce (10 nM to 5 µM), resulting in an EC_{50} of 0.47 ± 0.07 µM La and 0.19 ± 0.07 µM Ce. Experiments with Nd, exposures with various pH and hardness conditions, as well as exposures to mixtures of the three lanthanides of interest will be reported.

Copper and nickel bioaccumulation responses for two members of the *Hyalella azteca* cryptic species complex (PL)

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Hyalella azteca (Saussure, 1858), an amphipod crustacean, is frequently used in freshwater toxicity tests (e.g., metals, pH, organic contaminants, sediments). Since the mid-1980s, numerous organizations have collected and established cultures of *H. azteca* originating from localities across North America. However, *H. azteca* is actually a cryptic species complex whose members satisfy both the biological and the phylogenetic species concepts. As a result of morphological similarities among these lineages, they have been misclassified as a single species. To date, there are 85 clades that belong to the *H. azteca* species complex. Recent publications documented that certain members within the species complex have different toxicity responses to anions, an insecticide, and two metals. In the present study, two clades were delineated using DNA barcoding and exposed to increasing copper or nickel concentrations. A saturation model was used to determine the relationship between dissolved metal concentration in solution and in tissue. Bioaccumulation responses between clades for either copper or nickel exposure were not significantly different. These results indicate that although bioaccumulation is a good indicator of biological effect, the relationship between metal accumulation and mortality is complex. Nonetheless, the use of genetically characterized cultures of *H. azteca* is encouraged to maintain consistency between laboratories.

Assessing potential impact of land-applied biosolids to aquatic organisms (PL)

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Land application of biosolids is a key residuals management practice in Canada. Biosolids are utilized in crop fertility programs as a substitute for inorganic mineral fertilizers while also increasing soil organic carbon content. The resultant of municipal sludge treatment, biosolids also contain trace contaminants including Emerging Substances of Concern (ESOC) that may impact terrestrial and aquatic biota. Run-off from land and tile drainage is a key potential transport pathway for trace contaminants entering aquatic ecosystems. This study simulates land-application of biosolids and subsequent run-off and tile drainage water from rainfall events in a laboratory setting. Captured run-off and drainage water are subjected to a variety of lethal and sublethal aquatic bioassays with model organisms to study whole-organism impact.

Agrochemicals in the South Nation River watershed: Relative effects of atrazine versus nutrients on riparian and aquatic plants (PL)

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In agricultural watersheds, streams are intimately connected with croplands and may be inadvertently exposed to agrochemicals such as fertilizers and herbicides. Riparian and aquatic plants may be particularly affected by agrochemicals due to their taxonomic similarity to the intended targets (crop and weed species). Effects of agrochemicals on primary producers were assessed at 24 sites located throughout the South Nation River watershed, an agricultural watershed covering much of Eastern Ontario. Field sites ranged in surrounding agricultural land use (6.7-97.4% annual crops) and in-stream concentrations of reactive phosphate (4-102 $\mu g \cdot L^{-1}$) and nitrate (3-5404 $\mu g \cdot L^{-1}$). The impacts of herbicides on aquatic environments are difficult to assess with traditional grab sampling because concentrations are highly variable. An alternative approach using polar organic chemical integrative samplers (POCIS) provided time-weighted-average concentrations of the commonly used herbicide atrazine. Atrazine was widespread throughout the watershed with 56-day average concentrations ranging from 4-412 $\mu g \cdot L^{-1}$ and concentrations greater than 100 $\mu g \cdot L^{-1}$ at over half the field sites. Greenhouse concentration-response experiments provided evidence that the sensitivity of the aquatic macrophyte, *Lemna minor*, was lower in field populations previously exposed to atrazine. Vascular plant diversity was generally high throughout the watershed (285 riparian and aquatic plant species with species richness ranging from 43-107 species per site). However, the percentage of non-native plant species increased with increasing nitrate while the number of submerged species and overall floristic quality decreased. Results highlighted the need to reduce inputs of agrochemicals to streams and demonstrated

that effects of nutrients, specifically nitrate, generally superseded observable effects of the herbicide atrazine.

Assessing the chronic growth and reproductive effects of hydroxypropyl-β-cyclodextrin on the American flagfish (*Jordanella floridae*) over one full life cycle (PL)

Jordan Anderson[1], Lindsay Beyger[1], John Guchardi[1] and Douglas Holdway[1]

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Identifying the environmental risks of pharmaceuticals and personal care products (PPCPs) is a very important task in the field of aquatic toxicology. PPCPs are often designed to elicit a biological response in humans, but through genetic conservation, similar effects are often observed in non-target organisms, particularly fish. Hydroxylpropyl-β-cyclodextrin (HPβCD) is a toroidal-shaped amphiphilic compound with the ability to form non-covalent inclusion complexes with a variety of guest molecules. HPβCD can reduce volatility as well as improve the aqueous solubility of apolar guest compounds. As such, HPβCD has become an emerging PPCP, often used as a flavour and odour stabilizer or a deodorizer—for example, Febreze®. Additionally, HPβCD is extensively used as an excipient in the pharmaceutical industry. With the potential for entering the environment through wastewater treatment plant (WWTP) effluent, HPβCD poses an unknown risk to non-target aquatic biota. A chronic study using American flagfish (*Jordanella floridae*) was completed. Fish were exposed to flow-through concentrations of 0 – control, 5, 16, 50, 160, 500, and 1600 $\mu g \cdot L^{-1}$ of HPβCD maintained via a peristaltic pump for 145 d. Growth, condition factor (K), hepatosomatic index (HSI), and fecundity were monitored with no significant difference between treatments and controls ($p \leq 0.05$). A significant increase in the gonadosomatic index (GSI) was observed in females exposed to 5 and 50 $\mu g \cdot L^{-1}$ of HPβCD when compared to the controls ($p \leq 0.10$). Furthermore, when treatments were pooled and analyzed against the controls, a significant increase in GSI was observed ($p \leq 0.05$). An acute copper toxicity challenge assay was also completed on F1 flagfish larvae. Copper tolerance was significantly reduced in larval offspring from parents exposed to HPβCD. Future directions, including HPβCD's potential in a mixture setting, will be discussed.

Waterborne fluoxetine affects the stress and reproductive axes of developing zebrafish (PL)

Marilyn Vera Chang[1], Thomas Moon[1] and Vance Trudeau[1]

[1]University of Ottawa

The increase of pharmaceuticals present in the aquatic environment and the endocrine disruptive properties of at least some of these drugs have a potentially

detrimental effect on the aquatic life. The active ingredient of the antidepressant Prozac®, fluoxetine (FLX), is detected in aquatic environments at concentrations as high as 0.54 $\mu g \cdot L^{-1}$. Fluoxetine is a selective serotonin reuptake inhibitor that increases the extracellular brain levels of serotonin. Since serotoninergic systems are highly conserved, the presence of FLX in aquatic environments may result in unintended effects to aquatic vertebrates. We investigated the effects of environmental FLX concentrations on the stress and reproductive axes of zebrafish (ZF; *Danio rerio*). Embryos/larvae were exposed to either control or two concentrations of FLX: 0.54 $\mu g \cdot L^{-1}$ and 54 $\mu g \cdot L^{-1}$ from 3 hours post fertilization (hpf) to 6 days post fertilization (dpf). The stress axis was assessed by examining the physiological and behavioural responses following standardized net and novel tank stressors, respectively, at adulthood (6 months). Results indicate that FLX exposure of ZF embryo/larvae decreased basal and stress-induced whole-body cortisol levels at adulthood in a concentration-dependent manner ($p<0.05$). The FLX-treated fish displayed more anxiety-like behaviours compared to the control, with more severe effects observed at the lower concentration ($p<0.05$). A decrease in aromatase activity was also observed in ovaries exposed to the highest FLX concentration ($p<0.05$). The offspring of the FLX-treated parents exhibited a white-body phenotype, which was not observed in the controls ($p<0.05$). These results demonstrate that early life exposure of FLX disrupts the stress and reproductive systems of ZF. Fluoxetine also disrupts development in the offspring of exposed parents. Further studies will determine if the effects observed in the FLX-treated embryos/larvae persist across generations. Supported by NSERC.

Development of next generation bacterial bio-sensors for the detection of genotoxic and mutagenic activity of polycyclic aromatic hydrocarbons (PAHs), haloacetic acids (HAAs) and haloalkanes in environmental waters with enhanced correlation to human health risk (PL)

Will Lush[1] **and Kajaparan Jeyanthan**[1]

[1]Environmental Bio-Detection Products Inc.

Environmental Bio-Detection Products Inc.'s bacterial-based mutation assays ("Ames Test") and genotoxicity assays (umuC Assay and the SOS-ChromoTest Kit) currently use similar methodologies, which date back almost 40 years. Traditional and regulated methods for the bacterial tests outlined by the Organisation for Economic Co-operation and Development (OECD), the International Organization for Standardization (ISO) and other published guidelines require activation with an S9 liver fraction, which is usually prepared from rat liver post-mitochondrial supernatant fractions derived from rats that have been exposed to PCBs to activate specific liver enzymes. The S9 activation enzymes are an important aspect for bacteria toxicity tests, as bacteria use metabolic enzymes that differ greatly from those of mammals. The introduction of the S9 mix into tests allows for xenobiotic metabolic reactions that are similar to the ones found in mammals. This is a very crude and blunt approach which can often lead to compromised data. In the past year, a new generation of bacterial-based assays for the detection of

mutagenic and genotoxic activity has been developed in overcoming issues with the S9 mix. These assays have been designed in such a manner that the test bacteria express human P450s and GST liver enzymes, which are involved in mammalian xenobiotic bioactivation. This internal bio-activation process currently expresses both recombinant human P450 1A2 and GST T1-1 enzymes, and thus overcomes issues with traditional methods, in which (1) protein concentrations may vary between batches of S9 mixes, (2) false negatives may result from the potential sequestering of mutagens by S9 liver extract components (like fats and carbohydrates), and (3) short-lived reactive DNA mutagens are less likely to be detected in extracellular xenobiotic metabolic reactions. This next generation of human P450 1A2-expressing bacterial strains is designed to detect both mutagenicity and genotoxicty in Phase I bio-transformation (oxidation, reductive and hydrolytic reactions), whereas the GST T1-1 human-expressing strains are designed to detect Phase II (conjugation reactions). The development of these new bacterial-based bio-sensors will allow for greater sensitivity in the detection of emerging contaminants in the environment and greater correlation with potential human health effects.

Effect of herbicide surfactant polyethoxylated tallow amine (POEA) on production of egg masses and viability of eggs of rams-horn snail, *Planorbella pilsbryi* (PO)

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Polyethoxylated tallow amine (POEA) is a commonly used surfactant in formulations of the herbicide glyphosate. A number of studies have shown that POEA is more toxic to aquatic species relative to the active ingredient in the formulation, with the exception of primary producers. The present study examined the effect of POEA exposure on the rams-horn snail, *Planobella pilsbryi*, which is a common freshwater species widely distributed across Canada. In the primary stage of the study, adult snails were exposed to POEA at concentrations up to 40 $mg \cdot L^{-1}$ for 96 hours. Adult snails were removed and egg masses were incubated for 28 days to allow sufficient time for viable eggs to hatch. Mortality was not observed in concentrations ≤10 $mg \cdot L^{-1}$. A negative concentration-dependent trend in the production of egg mass was observed, resulting in an EC_{50} value of 10.5 $mg \cdot L^{-1}$. However, there were no significant differences between the viability of eggs in treatments exposed to POEA and the controls ($p>0.05$). In the secondary stage of study, adult snails were given 96 hours to lay egg masses without exposure to POEA. After 96 hours, adult snails were removed and POEA was added. The purpose of the change in experimental design was to determine whether the timing of exposure influenced the viability of eggs. Timing of exposure is an important consideration due to the relatively rapid dissipation of POEA in water. After 28 days of incubation, viability of eggs in treatments exposed to POEA was not significantly different from controls. The findings of this study suggest that exposure of freshwater snails to POEA may inhibit deposition of egg masses but not the viability of the eggs.

Sensitivity of aquatic species to Cúspide 480SL tank mix formulation of glyphosate used in control of coca in Colombia (PO)

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A number of different formulations of glyphosate have been used in the control of coca in Colombia over the last 15 years. In 2011, the government of Colombia began to use a new formulation, Cúspide 480SL. Cúspide 480SL contains a class of alkyl polyglycoside surfactant, which is different from the commonly used polyethoxylated tallow amine (POEA) found in products such as Roundup® and Vision®. The toxicity of previously-used spray mixtures to aquatic species has been reported in the literature but there were no data for Cúspide 480SL. This study investigated the toxicity of a mixture of Cúspide 480SL and the adjuvant CosmoFlux F411 (as applied in the field) to twelve aquatic species (*Pseudokirchneriella subcapitata, Lemna minor, Ceriodaphnia dubia, Daphnia magna, Hyallela azteca, Chironomus dilutus, Hexagenia spp., Lumbriculus variegatus, Planorbella pilsbryi, Pimephales promelas, Oncorhynchus mykiss, and Salvelinus namycush*). Organisms were exposed to the spray mixture at concentrations ranging from 0 to 500 mg glyphosate acid equivalent (a.e.)·L^{-1} for 48 or 96 hours, depending on the species. EC_{50} values greater than 150 mg a.e.·L^{-1} were observed for eight of the twelve species, indicating that spray mixture is less toxic than the previous spray mixture to these eight species. The spray mixture was also found to be less toxic to *C. dubia* and *D. magna* relative to previous mixtures. Similar toxicity was observed in *P. subcapitata* and *L. minor* relative to formulations containing POEA. The findings of the study indicate that, compared to formulations of glyphosate used in the control of coca in the past, spray drift from the aerial application of the spray mix made from Cúspide 480SL may pose less risk to aquatic ecosystems.

Developments in derivation of water quality guidelines for metals (PO)

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[1]Environment Canada

Environment Canada develops environmental quality guidelines for water, sediment, tissue residue, and soil through Canadian Council of Ministers of the Environment (CCME) and also federally (the Federal Environmental Quality Guidelines (FEQGs)). Over the last five years the National Guidelines and Standards Office (NGSO) has developed water quality guidelines for the protection of aquatic life for various toxic substances and is evaluating the latest tools and approaches for considering toxicity modifying factors (TMFs) in guideline derivation for metals. The aquatic toxicity of metals can be influenced by physical and chemical characteristics of the water that can change bioavailability; these can include water hardness, pH, and dissolved organic carbon.

Consideration of these toxicity factors is used, where possible, to develop water quality guidelines of variable concentration that still provide the same intended level of protection to aquatic life. Single-variable regression analysis was used in the past to account for individual TMFs. We are now examining options for incorporating multiple TMFs. Guidelines that account for toxicity modifying factors are represented by equations and/or models and allow users to input local water chemistry to calculate site-specific water quality guidelines.

Disruption of collagen deposition *in vitro* as a potential chronic toxicity assay (PO)

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Persistent or progressive deterioration of cells, tissues and organisms is the result of chronic toxicity from long-term exposure to chemicals. Prevalence of skeletal deformities has been used as a bioindicator of chronic pollution, and fish deformities and growth reduction are parameters evaluated in chronic tests. Collagen is a major structural protein whose synthesis and deposition is pivotal for both structural integrity and growth. An *in vitro* assay evaluating contaminant effects on fish collagen synthesis, rapidly and inexpensively, could reduce the need for whole fish testing. YPF5, a novel Yellow Perch (*Perca flavescens*) cell line, was used to evaluate the *in vitro* effects of environmental contaminants on collagen synthesis and deposition. YPF5 is a fibroblastic cell line derived from the caudal fin of an adult feral perch specimen. The cells, in the presence of ascorbic acid, form distinct collagen fibrils detectable by immmunofluorescence. Addition of sample chemicals including cortisol, crude naphthenic acid or copper sulphate at sublethal doses disturbed the normal deposition of collagen. Within limits, YPF5 collagen synthesis could be used as a bioindicator and predictor of fish growth to reduce the need of whole fish testing in chronic toxicity assays.

Adverse Outcome Pathways: Linking Toxicity-related Molecular and Cellular Responses with Physiology and Ecology

Predicting the sensitivity of endangered sturgeons to dioxin-like compounds: Molecular investigation into the aryl hydrocarbon receptor pathway (PL)

Jon Doering[1], Reza Farmahin[2], Steve Wiseman[1], Shawn Beitel[1], Sean Kennedy[2], John Giesy[1] and Markus Hecker[1]

[1]University of Saskatchewan, [2]Environment Canada

Numerous sturgeon species around the world are endangered, which has rendered these fishes of great interest in connection with the risk assessment of anthropogenic stressors. However, current risk assessment attempts are hampered by a lack of knowledge about the sensitivity of sturgeons to toxicants of concern, such as dioxin-like compounds (DLCs). DLCs elicit their toxic action through activation of the aryl hydrocarbon receptor (AhR) and are known to cause a variety of adverse effects in vertebrates. In birds, the amino acid sequence of the ligand binding domain of the AhR is known to result in differences in affinity for DLCs and therefore differences in sensitivity among species. In order to incorporate differences in species sensitivity of sturgeons to DLCs into the adverse outcome pathway framework, the objectives of this study were to: (a) characterize the relative differences in sensitivity to DLCs between two species of endangered sturgeon (white sturgeon and lake sturgeon); and (b) develop methods to predict the relative sensitivity of other species of sturgeons to DLCs based upon differences in mechanisms of toxicity. White sturgeon were found to have six-fold greater response based on up-regulation of hepatic cytochrome P4501A (CYP1A) transcript abundance following exposure to a model DLC (β-naphthoflavone), compared to lake sturgeon. Since effects of exposure to DLCs are known to be driven by activation of the AhR, the AhR1 and AhR2 of white and lake sturgeon were sequenced and characterized for their activation potential by prototypical DLCs in an attempt to explain the observed differences between these species. White and lake sturgeon AhR1s had similar sensitivity to 2,3,7,8-TCDD *in vitro* by use of the luciferase reporter gene assay and transfected COS-7 cells, while white sturgeon AhR2 had an EC_{50} 10-fold less than lake sturgeon AhR2. Relative differences in response between white and lake sturgeon *in vivo* and with regard to AhR2 activation *in vitro* appear similar, indicating that the AhR2 might drive differences in sensitivity to DLCs among these species of sturgeons. Homology modelling will be used to predict whether amino acid differences in the ligand binding domain of the AhR2 drive the observed differences in sensitivity between the AhR2 of white and lake sturgeon, and could allow prediction of the sensitivity of other endangered sturgeon species based on

their AhR2 amino acid sequence. This research could be essential in the risk assessment of sturgeons, and other endangered fishes, to DLCs.

Does the intracellular partitioning of non-essential metals represent a toxicological risk for *Anguilla anguilla* and *Anguilla rostrata* eels inhabiting the St. Lawrence and Gironde estuaries? (PL)

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The populations of American (*Anguilla rostrata*) and European (*Anguilla anguilla*) eels have severely declined over the last 30 years. In addition to other factors, the metal contamination that occurs in the St. Lawrence and Gironde estuaries may cause deleterious effects in eels inhabiting these aquatic environments. In this context, metal-induced toxicity can be caused by the inappropriate binding of metals to physiologically sensitive target molecules (e.g., cytosolic enzymes) and organelles (e.g., mitochondria). With this in mind, we determined the subcellular partitioning of silver (Ag), arsenic (As), cadmium (Cd), nickel (Ni), thallium (Tl) and lead (Pb) in livers of American and European yellow eels collected in the St. Lawrence and Gironde estuaries, respectively. A subcellular partitioning procedure using differential centrifugation, NaOH digestion and thermal shock steps was applied to separate liver samples into putative metal-sensitive fractions (heat-denatured proteins, mitochondria and microsomes/lysosomes) and detoxified metal fractions (heat-stable proteins and NaOH-resistant granules). The trace metals were measured in eel homogenates and in each subcellular fraction by inductively coupled plasma-mass spectrometry (ICP-MS). Differences in subcellular metal-handling strategies were observed among metals and between species. Our results also revealed that even though the eels possessed metal-detoxification strategies that sequestered and immobilized these potentially toxic metals, these mechanisms were not completely efficient in preventing metal binding to some sensitive sites in the intracellular environment, mainly mitochondria (e.g., As, Cd and Tl) and microsomes/lysosomes fractions (e.g., As and Ni). We suggest that metal contamination in the yellow eels studied could lead to deleterious effects and contribute to the observed population decline.

Is oxidative stress the main driver of selenium toxicity in juvenile white sturgeon (*Acipenser transmontanus*)? (PL)

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Many species of sturgeon have experienced significant population declines and poor recruitment over the past decades, which have led to many populations being listed as endangered. While the reasons for these declines are not yet fully understood, the benthic lifestyle, longevity, and delayed sexual maturation of many sturgeons likely render them particularly susceptible to factors such as habitat alteration and exposure to contaminants. Toxicity studies have shown white sturgeon (*Acipenser transmontanus*) to be one of the most sensitive species of fish to pollutants such as metals and dioxin-like chemicals. Selenium (Se) is of particular concern, especially in its more bioavailable form selenomethionine (SeMet). It is hypothesized that increased releases of Se to aquatic environments have contributed in part to sturgeon declines; however, to date little is known about its specific effects. Therefore, the aim of this study was to investigate the toxicity of dietary SeMet to juvenile white sturgeon and to link physiological effects to key molecular events of toxicity. Oxidative stress in liver tissue was focused on as it was hypothesized to be a primary mode of toxicity. Specifically, 4-year-old white sturgeon were exposed for 72 days to 1, 5, 25 or 100 µg SeMet (dm) per g food. Doses were chosen to range over a necessary Se intake level, current environmentally relevant intakes and an intake representing predicted increases of Se release. Se was found to accumulate in a dose-dependent manner in tissues. Histology of the liver showed a significant and dose-dependent increase in macrophage aggregations and decrease of energy stores and cell size. Results of a lipid hydroperoxide assay were not significant, which shed doubt on oxidative stress being the main driver of toxicity. Real time PCR was used to examine changes in oxidative stress markers. A full transcriptome analysis using Illumina MiSeq technology was conducted to elucidate the molecular mode(s) of toxicity of SeMet. Approximately 2000 transcripts were down-regulated and 900 up-regulated by more than two-fold. Taken together the data makes a strong case for the sensitivity of white sturgeon to Se accumulation and indicates a general suppression of health due to toxic levels of exposure. Ongoing analysis of unbiased gene expression data will shed light on the specific pathways by which SeMet affects white sturgeon.

Dietary selenium affects growth and hepatic cell signalling cascades in juvenile rainbow trout (PL)

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Selenium is an essential trace element that can be mobilized into aquatic environments by mining activities. Dietary selenomethionine (SeMet) treatments have been observed to have adverse effects on adult fish, and can alter growth and energy stores. However, the effect of Se on juvenile fish, a potentially more sensitive life stage, has yet to be fully explored. The objectives of this study were to determine the effects of dietary SeMet on energy stores and molecular pathways in juvenile rainbow trout liver. Juvenile rainbow trout were fed *Lumbriculus* worms cultured in different concentrations of SeMet-supplemented yeast (control or treatments consisting of target concentrations of 5, 10, 20 or 40 $mg \cdot kg^{-1}$ Se dry weight) for 60 days, and effects on growth and liver triglycerides, glycogen, total glutathione, 8-isoprostane and gene expression were measured. It was determined that fish reached steady state concentrations of whole body Se between 15 and 30 days. Fish fed 20 and 40 $mg \cdot kg^{-1}$ Se dry weight had significantly lower length and weight, and significantly lower hepatic triglyceride levels, compared to control fish. Genes, measured by real-time PCR, involved in metabolism and energy storage (e.g., fatty acid synthase, ATP citrate lyase, glucose-6-phosphate dehydrogenase, citrate synthase and acetyl-CoA carboxylase) did not significantly differ among groups. Microarray analysis suggested that genes involved in cell growth and proliferation were affected in individuals fed all four doses of Se. Other pathways affected by Se included those involved in epidermal growth factor receptor signaling, growth hormone receptor and insulin growth factor receptor 1 signaling. Most interesting was that growth hormone receptor 1 mRNA levels were significantly increased in the liver of trout at all four doses. Additionally, gene sets involved in fatty acid beta-oxidation, fatty acid transport and regulation of fatty acid oxidation were affected in the livers of individuals fed 40 $mg \cdot kg^{-1}$ Se. This study suggests that chronic, environmentally relevant levels of dietary SeMet exposure can alter energy storage as indicated by both the gene expression and triglycerides analyses, and this was associated with decreased size at the two highest doses examined. These results suggest that dietary Se may impair growth and development during sensitive early developmental stages of salmonids. This study provides a unique opportunity to link pathways altered by Se to higher-level endpoints such as growth and energy homeostasis, which will lead to the development of an adverse outcome pathway.

The potential of selenium to counteract arsenic-induced inflammation and vascular damage (PL)

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Arsenic (As) is a widespread and growing contaminant in drinking water. Millions of people who are chronically exposed to As are at risk of developing serious diseases such as cancers, peripheral vascular and cardiovascular diseases. Selenium, an essential trace element with anti-oxidant properties, is a well-known antagonist of As *in vivo*. In the liver and circulation of mammals, arsenite and selenite, together with glutathione, form a seleno-bis(S-glutathionyl) arsinium ion, $[(GS)2AsSe]^-$, which can be excreted in the bile. In an experimental study on mice, we found that atherosclerosis triggered by moderate levels (200 $\mu g \cdot L^{-1}$) of As in the drinking water was reduced by dietary Se consumed in form of naturally Se-rich lentils. In an effort to elucidate the mechanisms of actions involved in As-related atherogenesis, and the effect of selenium supplementation on this inflammatory process, we examined the effects of As and Se exposure in human aortic endothelial cells (HAEC) and leucocytes. Here, we proved that As increased the expression of the inflammatory mediator, IL-8 in HAEC, which was inhibited by addition of selenite. Wound healing capacity of HAEC was greatly impaired when cells were incubated with 5uM As. The inhibited wound healing was almost completely reversed by co-incubation with selenite or selenomethionine. In As-induced inflammatory processes, we examined leucocyte recruitment under physiological shear stress and As-dependent expression of adhesion molecules in HAEC, which are crucial for leucocyte extravasation into the tissue and formation of atherosclerotic lesions. We discuss how our research offers insight into the role of subclinical levels of As on the inflammatory response, and how Se may offer protection against As-induced damage.

Linking sublethal neuroendocrine effects of contaminants on the hypothalamic-gonadal-pituitary axis to adverse outcomes in fish (PL)

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Linking key molecular initiating events to adverse outcomes is an important component of the adverse outcome pathway (AOP). An AOP that relates mechanistic data to individual-level adverse reproductive outcomes in order to predict population-level impacts is currently being developed. The hypothalamic-pituitary-gonadal axis (HPG) is critical in the development and regulation of an organism's reproductive system. Many end products of the hormonal cascade that occur within the fish HPG are controlled by

the upstream release of the neurotransmitters gamma-aminobutyric acid and dopamine. Molecular initiating events within the HPG are likely to arise, as many toxicants have been known to cause inhibition of neurotransmitter receptor binding, enzyme activity and uptake transportation. Previous models that have been developed to simulate the fish HPG axis have not included a neurochemical compartment, which is critical in understanding how contaminants disrupt the upstream components of the HPG axis. Therefore, we adapted a current fish vitellogenesis model and incorporated a neurochemical compartment to better estimate reproductive effects caused by neurotoxicant exposure. This model integrates neurochemical results from high-throughput cell-free assays with the changes in an individual's vitellogenin production. Results of the model indicate that exposure to toxicants that interfere with neurotransmission can cause harmful reproductive effects. Our model outlines an approach that can be used to extrapolate from *in vitro* screening assays to predict changes within a population.

Characterization of methylmercury-associated neurochemical disruption in yellow perch (PL)

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Methylmercury (MeHg) is a ubiquitous contaminant that bioaccumulates in fish tissues and biomagnifies in aquatic ecosystems. Over the past decade many studies have shown that MeHg exposure can affect fish reproduction and lead to deleterious impacts on fish populations. While it is established that fish reproduction is controlled by the Hypothalamus-Pituitary-Gonad (HPG) axis, the effects of MeHg on key neurotransmitters and neurohormones modulating HPG function, such as GABA, dopamine, estrogen, and glutamate, are not well characterized. In this study we utilized yellow perch (*Perca flavescens*) as a model species. *In vivo* and *in vitro* studies were conducted to characterize which neurochemicals are affected by MeHg exposure. First, perch were exposed in the laboratory to different dietary MeHg concentrations (food pellets containing 0, 0.5, 5 and 50 $\mu g \cdot g^{-1}$, n=12 fish per condition) at different times of the fall reproductive period, to take into account the reproductive stage of fish. Neurochemicals investigated included GABA, D2 and NMDA receptor binding, and monoamine oxidase and glutamine synthetase enzyme activities in four different brain regions (brain stem, hypothalamus, telencephalon, optical tectum). Additional analyses were performed on livers and gonads to assess estrogen receptor binding. Levels of Hg in fish muscle exposed to 50 $\mu g \cdot g^{-1}$ were 186 times higher than controls. Estrogen receptor levels were statistically decreased in telencephalon and optical tectum of fish exposed to 50 $\mu g \cdot g^{-1}$ MeHg. Data on other neurochemicals are currently being compiled. Second, *in vitro* screening assays were

conducted to see if MeHg could directly interact with (and impair) receptors and enzymes important in fish reproduction and neurobehaviour. Adult yellow perch brains and livers were exposed *in vitro* to MeHg concentrations (0-320 µM). Results demonstrated inhibition of glutamate uptake and glutamine synthetase in brain extracts exposed to 54.7 ± 4.1 µM and 4 ± 2.5 µM MeHg respectively. The *in vitro* and *in vivo* studies here provide information that will help us better understand the mechanisms by which MeHg may impair fish neurobehaviour and reproduction.

Mechanisms of reproductive inhibition in zebrafish exposed to ethinylestradiol and ammonia (PL)

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As the global population rises, water demand increases concomitantly. Anthropogenic uses for water, including agricultural watering and residential sanitation, result in the accumulation of traceable levels of contaminants which require subsequent treatment methods for removal. Current municipal treated wastewater effluents (WWE) are deemed safe by government legislation, yet they remain a significant source of contaminants in aquatic ecosystems and these have been shown to induce a variety of physiological responses in exposed organisms. Many studies examining fish have reported significant effects of WWE on reproduction, including increases in body condition and liver somatic index, reductions in gonadal somatic index, increased occurrence of intersex condition in males, altered sex ratios, and decreased egg production. Given the chemical complexity of WWE, the issue of determining which constituents are affecting exposed organisms is exacerbated by the potential for multiple compounds to cause synergistic and/or antagonistic effects. The disconnect between single-contaminant exposures and exposure to complex mixtures is difficult to bridge, but can be approached by methodically adding additional compounds to single-contaminant exposures. This research aims to further the current understanding of the interactive effects of ethinylestradiol (EE2) and ammonia (NH_3), two physiologically-active constituents commonly found in WWE, using the zebrafish (*Danio rerio*) as a model. EE2 is the synthetic estrogen used in oral contraceptives, and NH_3 is a common nitrogenous waste produced by vertebrates. The current research sought to determine how chronic exposure to EE2 and NH_3 affects zebrafish reproduction. Zebrafish were exposed to EE2 and NH_3, both separately and combined, to study the effects on fecundity and reproductive physiology. These studies showed that when tested individually, both EE2 and NH_3 inhibit egg production. Additionally, exposure to EE2 causes a significant decrease in female gonadal-somatic index (GSI) and ovarian testosterone (T) and estradiol (E2) content. Although NH_3 alone does not decrease ovarian T or E2, when combined with EE2, sex-steroid content decreases beyond the reduction seen in the EE2 exposure. Interestingly, the combination of NH_3 to EE2 reverses the reduction in GSI associated with EE2 exposure. These findings suggest that the mechanistic inhibition of egg production

associated with EE2 and NH_3 exposures occurs through different pathways, and that endpoints such as GSI can be generic indicators of reproductive inhibition, providing little indication of the causative factors. Further studies of these interactive effects are needed in order to better apply the findings to the environment.

What does it cost to defend against chemical contaminants? P-glycoprotein induction and its energetic costs in rainbow trout (*Oncorhynchus mykiss*) (PL)

Chris Kennedy[1], Grant Fitzpatrick[1] and Braedon Cashion[1]

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Biological systems are constantly challenged by chemical toxicants and can utilize a variety of mechanisms to neutralize, repair, or reduce the potential for toxic effects. These various chemical defense mechanisms all utilize energy, but their specific costs and impacts on animal energy budgets are currently unknown. In this study, induction of the efflux transporter P-glycoprotein (P-gp; [ABCB1, MDR1]) and the associated energetic costs of substrate transport were examined. Intraperitoneal injection of chlotrimazole (0, 0.1, 1.0 and 10 $mg \cdot kg^{-1}$) increased P-gp activity (as measured in a competitive rhodamine 123 assay in hepatocytes) in a dose-dependent manner reaching a maximum induction of 2.8-fold. At 10 $mg \cdot kg^{-1}$ clotrimazole, P-gp induction was significantly higher than controls by 50 hours (1.3-fold) following inducer administration, which remained elevated until the last sampling time point at 168 hours. *In vitro* measurements of hepatocyte respiration indicated that basal P-gp activity using R123 as a substrate did not significantly alter respiration rates (range 18.0 to 23.2 ng O_2/minute/106 cells); however, following induction of P-gp by chlortrimazole and exposure to the P-gp substrate R123, respiration rates increased significantly (3.52-fold). In whole animal respirometry, fish exposed only to R123 or induced with clotrimazole did not differ from control fish respiration rates (range 1.2 to 2.1 mg O_2/kg/minute), but fish with induced P-gp levels and then exposed to the P-gp substrate R123 exhibited significantly increased respiration rates. This work indicates that basal and induced levels of P-gp activity do not incur significant energetic costs to fish; however, upon induction of P-gp and substrate exposure, energetic costs are significant and may pose challenges to organisms facing limited energy resources.

Aryl hydrocarbon receptor adverse outcome pathway for assessing toxic effects of dioxin-like compounds in birds (PO)

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Adverse outcome pathways (AOPs) are a conceptual framework used to collect, organize and evaluate the relevant information available on a chemical and its biological

and toxicological effects. Development of an AOP involves linking a molecular initiating event (MIE) to an adverse outcome at a biological level relevant to risk assessment through a series of key events occurring at different levels of biological organization. In 2012, the Organisation for Economic Co-operation and Development (OECD) launched their program for the development and review of AOPs. The process includes submission of a proposal by a member country that, once accepted by the OECD, is included in the AOP development workplan. The AOP can then be developed using the AOP Wiki platform and other components of the AOP knowledge base, which allow for input by the public and OECD expert groups. When the AOP has been sufficiently characterized, a descriptive document is prepared by the project lead and goes through a series of reviews. After receiving the endorsement of all groups involved in the review process, the AOP is published by the OECD. The AOP may also be used to develop test guidelines and integrated testing strategies, and can be incorporated in the OECD Quantitative Structure-Activity Relationship (QSAR) Toolbox.

A number of adverse effects, including developmental abnormalities uroporphyria and embryolethality, have been observed in birds following exposure to dioxin-like compounds (DLCs). There is considerable evidence that these effects occur subsequent to activation of the aryl hydrocarbon receptor (AHR). In 2013, "The Adverse Outcome Pathway for Sustained Activation of the Avian Aryl Hydrocarbon Receptor" was included in the AOP development workplan. The AOP describes the relationship between activation of the avian AHR by DLCs (MIE) and the adverse outcomes of cardiotoxicity, hepatotoxicity and embryotoxicity in birds. The key events linking the MIE to embryotoxicity and potential population declines include induction of phase I and phase II enzymes, changes in hormone levels, deregulation of AHR nuclear translocator-dependent pathways, and effects on organ development and function. The relationship between AHR activation and uroporphyria is very well characterized. Key events include induction of cytochrome P450 1A5, uroporphyrinogen oxidation, and accumulation of highly carboxylated porphyrins in the liver, kidneys, bones and blood. Using the recently published OECD guidance, the experimental research supporting the relationships between the MIE, key events, and adverse outcome was evaluated using the proposed weight-of-evidence classification scheme, and the overall AOP was assessed using the Bradford Hill criteria. This AOP has been added to the AOP Wiki and a first draft of the descriptive document was recently submitted to the OECD for review.

Temporal- and concentration-dependent effects of polycyclic aromatic hydrocarbon phenanthrene on the transcriptome of the fathead minnow (*Pimephales promelas*) (PO)

Jennifer Loughery[1], Angella Mercer[1], Karen Kidd[1] and Christopher Martyniuk[1]

[1]University of New Brunswick

Phenanthrene (PHEN) is a polycyclic aromatic hydrocarbon that is found in the environment at relatively high concentrations and is associated with anthropogenic

activities in addition to natural sources of coal, oil and tar deposits. Female fathead minnow (FHM; *Pimephales promelas*) were exposed to waterborne concentrations (average measured 0, 29.8, 389.4 and 942.5 $\mu g \cdot L^{-1}$) of PHEN over 72 hours. Tissue samples were collected at 24hr periods from each treatment. Gonadal tissues were incubated for 24 hours and sex steroid production was measured *in vitro*. Microarray analyses were performed on liver tissues in two experiments: (a) individuals that were exposed to one of three PHEN concentrations or a control (at the 48-hour time point); and (b) individuals that were exposed to 29.8 $\mu g \cdot L^{-1}$ PHEN over three different time points (24, 48, and 72 hours). 17β-estradiol (E2) production was analyzed in individuals from both experiments. There were 745, 764 and 1585 differentially expressed gene probes after PHEN treatments of 29.8, 389.4 and 942.5 $\mu g \cdot L^{-1}$, respectively, in the 48hr experiment, and there were 137 gene probes in common across the three concentrations.

Hierarchical clustering of differentially expressed genes showed that, in general, individuals from the control and low PHEN treatment grouped together while individuals treated with medium and high PHEN concentrations grouped together. Parametric Analysis of Gene Set Enrichment (PAGE) analysis suggested that gene ontology terms associated with insulin receptor binding and insulin growth factor receptor signalling pathways were affected by the low PHEN concentration; heat shock protein and lipid metabolic processes were affected by the medium PHEN concentration; and thyroid hormone receptor binding and lipid transport were affected by the high PHEN concentration. Of the significant GO terms, only 8 were similar between concentrations, and these were related to DNA and RNA replication and carbohydrate biosynthesis. The numbers of differentially expressed genes related to the timing of the exposure were 349, 635, 772 after 24, 48 and 72 hours, respectively. Of the differentially expressed genes, only four were similar between time points (cyclin-dependent kinase11B, methyltransferase7A, cytochrome P450 27B1 and aryl hydrocarbon receptor nuclear translocator). PAGE analysis suggested that gene ontology terms associated with lipid binding and monoxygenase activity were affected after 24 hours; heat shock protein binding and insulin receptor binding were affected after 48 hours; and oxidoreductase activity, lipid binding, and monooxygenase activity were affected by PHEN after 72 hours. E2 production was not different from controls at any time point and there were no differences in E2 levels with dose after 48 hours. This research suggests that both dose and time of exposure can result in unique transcriptional responses to PHEN in the FHM liver, and more in depth studies should focus on the temporal responses of the transcriptome.

The neuroactive pesticide dieldrin affects embryonic development of zebrafish (PO)

Kathleena Sarty[1], Andrew Cowie[1] and Christopher Martyniuk[2]

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The organochlorine pesticide dieldrin was used as an alternative to aldrin in agriculture from the 1950s to mid-1980s. Dieldrin is an effective pesticide because it antagonizes γ-aminobutyric acid receptors, the primary inhibitory neurotransmitter system in the central nervous system, and results in over-excitation and death. However, due to human health concerns (it has been associated with neurological diseases), the use of this pesticide was discontinued in North America. Despite the fact that it is considered a legacy pesticide, dieldrin remains present in some agricultural regions in sediment, is still detectable in some drinking water sources, and readily bioaccumulates in fatty tissues of benthic organisms (i.e., embryos). In this study, we examined the survival, hatch rate, morphological, and behavioural effects of chorionated and dechorionated zebrafish (*Danio rerio*) after a 48 hour exposure to dieldrin at concentrations ranging from 1.32 x105 $ng \cdot L^{-1}$ to 1.32 x107 $ng \cdot L^{-1}$. Chorionated embryos exposed to 1.32 x107 $ng \cdot L^{-1}$ of dieldrin in 1% DMSO showed less than 3% mortality for all embryos after 48 hours. Dechorionated fish exposed to 1.32 x107 $ng \cdot L^{-1}$ of dieldrin in 1% DMSO were more sensitive to dieldrin and displayed higher mortality (32%) than chorionated embryos. Dieldrin had no detectable effect on hatch rate and did not show any effects on growth-related endpoints. However, dieldrin exhibited delayed toxicity in embryos, with 25% survival of chorionated fish and 33% survival of dechorionated fish after a 6-day depuration period in the highest treatment group. After a depuration stage, morphological deficits of cardiac edema, cardiac hemorrhaging, skeletal deformities, and lethargy were noted in animals treated with dieldrin. Using mechanistic data that will be collected, this study contributes to the development of an adverse outcome pathway for GABAA receptor antagonism during zebrafish development.

Morphological responses of embryonic zebrafish (*Danio rerio*) to the pesticide fipronil (PO)

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The phenylpyrazole fipronil is a commonly used insecticide designed to inhibit γ -amino-butyric acid (GABA) receptors, the dominant inhibitory neurotransmitter in the central nervous system. Fipronil in the environment has been detected between 1-2 $ng \cdot L^{-1}$ and 10 $\mu g \cdot L^{-1}$ from agricultural and urban runoff. Although designed to target $GABA_A$ receptors in insects, fipronil can also target the GABAergic systems of fish, and this possesses a risk to the health of fish that inhabit fipronil-contaminated environments. We investigated the persistent morphological responses of fipronil on embryos using the

zebrafish (*Danio rerio*) model due to their strength for embryogenesis. Three-hour-old zebrafish embryos were exposed to either a concentration of fipronil (ranging from 0.02 $ng \cdot L^{-1}$ to 4000 $ng \cdot L^{-1}$), a solvent control (0.01% ethanol) or rearing medium control. Following a 48-hour exposure, embryos were transferred to clean rearing medium for 7 days. Embryos exposed to 2000 $ng \cdot L^{-1}$ and 4000 $ng \cdot L^{-1}$ fipronil displayed significantly higher mortality, reduced body length and an increase in lethargy compared to controls, while embryos exposed to a concentration of 0.02 $ng \cdot L^{-1}$ displayed shortened notochord length. This study suggests that fipronil at environmentally relevant concentrations does not affect the survival or morphology of zebrafish embryos. However, sublethal endpoints such as gene expression for genes related to the GABA system will be examined to characterize potential sublethal effects of fipronil exposure.

Seasonal variation in the sensitivity of larval sea lampreys to the pesticide 3-trifluoromethyl-4-nitrophenol (TFM), used to control invasive sea lamprey in the Great Lakes (PO)

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Sea lamprey (*Petromyzon marinus*), a parasitic jawless vertebrate which uses its oral disk to feed on the blood of host fishes, are invasive to the Great Lakes. The chemical 3-trifluoromethyl-4-nitrophenol (TFM) is widely used to control sea lamprey populations because it is selectively toxic to larval sea lampreys. Sensitivity to TFM in larval sea lamprey varies seasonally, but the underlying causes are not understood. The objective of this research was to: (a) determine the relative contributions that seasonal variations in energy stores, body size and water temperature play in explaining these differences in TFM sensitivity; and (b) identify the underlying causes for any differences in TFM sensitivity. Accordingly, larval sea lampreys were collected in the spring, summer and fall and returned to the Hammond Bay Biological Station on Lake Huron, where they were held at the same temperature as their natal streams. Following toxicity tests, whole-body TFM concentrations and energy stores were quantified. We conclude that there is a seasonal variation in TFM sensitivity, and a combination of low glycogen stores and lower body mass explains the greater sensitivity of larval sea lampreys to TFM. Thus, lower concentrations of TFM may be effective if applied in the spring, but higher doses may be needed later in the year, when the animals are larger and have greater energy stores.

Chemical and Biological Effects of Data-poor Metals and Metal Mixtures

Hexavalent chromium (CrVI), the newcomer in northern Ontario: Interactive effects of CrVI and calcium on the survival and reproduction of *Daphnia pulicaria*, a common zooplankter in northern Ontario lakes (PL)

Martha Celis-Salgado[1], John Bailey[2] and Norman Yan[1]

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The development of the Ring of Fire chromite ore bodies may dramatically increase chromium levels in northern Ontario lakes. Because the form of chromium (Cr) varies with environmental conditions, and northern Ontario lakes vary in calcium (Ca) concentrations, we tested the toxicity of hexavalent CrVI to a common, native soft water daphnid species along a Ca gradient, which reflects the range of hardness in northern Ontario lakes. We ran 14-day chronic, with 48-hour renewal bioassays, using <24-hour *Daphnia pulicaria* neonates, in a chemically defined soft water medium (FLAMES medium) with a range of Ca levels of 1.5 $mg \cdot L^{-1}$ to 10.0 $mg \cdot L^{-1}$, and CrVI levels of 1.0 $\mu g \cdot L^{-1}$ to 4.0 $\mu g \cdot L^{-1}$. CrVI nominal concentrations were confirmed by IC-ICP-MS. The toxicity of CrVI increased with the Ca content in the media. Reproduction was negatively affected by Cr at higher Ca concentrations, as evidenced by an increasing number of dead neonates. Unlike other metals, raising Ca did not protect daphnids from the effects of Cr. The 14-day LC_{50} of CrVI for *D. pulicaria* we observed is near the Canadian Water Quality Guideline for the protection of aquatic life (freshwater), meaning the guideline is likely too high to protect this common daphnid in soft water lakes. This study provides insights into the varying effects of CrVI on *D. pulicaria* in soft water and unexpected responses along a relatively short, but environmentally relevant Ca gradient.

Effects of lithium upon mineral metabolism in juvenile rainbow trout (PL)

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Increased use of lithium (Li) for manufacturing and medicinal purposes may cause increased risk of water contamination, and the knowledge about effects of Li on freshwater aquatic organisms is very limited. A complex approach to investigate Li effects

on juvenile Rainbow trout blood plasma and brain in a controlled laboratory experiment included quantitative target analysis of common ions (blood plasma and brain) and qualitative metabolomics analysis (plasma only). Fish were exposed to sublethal Li concentrations around 1 mg $Li \cdot L^{-1}$ versus control >2 µg $Li \cdot L^{-1}$. Water quality parameters (dissolved oxygen, pH, conductivity, ammonia, temperature) were measured throughout the exposure period and only total ammonia and ammonia ion changed. Changes occurred in the plasma proteome that corresponded to changes in plasma Li concentration for exposed fish. These changes include inhibition of prostaglandin synthase (Ptgs2), copper transporting ATP synthases, effects on the transport of lipid molecules (LDL), and up-regulated expression of Na/K transporting ATPase. There were also changes in concentrations of major cations, and free fatty acids involved in osmoregulation. Fish brain showed ion loss for sodium, calcium, and potassium and decline in ammonium cation. In plasma, sodium and calcium ions decline was accompanied by an increase in potassium, magnesium and ammonium concentrations. This study demonstrated exposure to elevated Li concentration in fish brain and plasma caused inhibition of Ptgs2 followed by elevation of Arachidonic Acid (AA), potassium and magnesium ion concentrations, and corresponding loss of sodium and calcium ions. We suspect that these changes represent an attempt by the fish to maintain homeostasis in fresh water. Thus, these results suggest that Li may be an ionoregulatory toxicant in the aquatic environment, via Li-induced inhibition of Ptgs2 and possible interaction between Li and copper transporting ATP synthases.

Comparative bioaccumulation and toxicity of sodium selenite and sodium selenate in the aquatic epibenthic invertebrate *Hyalella azteca*: Can bioaccumulation and toxicity be predicted and included in a metal mixture model? (PL)

Warren Norwood[1], Lesley Milne[2] and D. George Dixon[2]

[1]Environment Canada, [2]University of Waterloo

Selenium, even though it is a nonmetal, does show some metallic characteristic and is frequently found in metal sulfide ores, especially copper ores. Therefore, through mining and smelting processes, selenium can be a contaminant in aquatic systems. However, all animals require selenium for cellular function. Therefore, an understanding of the bioaccumulation and possible toxicity of selenium is required. Selenium occurs naturally in two major forms, selenite and selenate, hence the bioaccumulation and toxicity of these two forms was assessed. The relationship between exposure, bioaccumulation and toxicity of these two forms was determined in hard water (Lake Ontario). Since selenium is associated with metal mining and smelting processes, it can be found in mixtures of metals and therefore its inclusion in a metal mixture assessment model would be useful. This possibility will be explored during the presentation.

A failure of biotic ligand model in predicting a trivalent metal bioaccumulation at the presence of organic ligands (PL)

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Rare earth elements (REEs) have numerous promising applications in the high-tech and clean energy industries, but their interactions with aquatic biota are still largely unknown. The bioavailability of several REEs has been carefully examined for the freshwater alga, *Chlamydomonas reinhardtii*, under the framework of the biotic ligand model (BLM). For example, the thulium (Tm) internalization flux (J_{int}) in algae increased linearly below the concentration of 1×10^{-7} M before reaching saturation. The calculated maximum internalization rate (J_{max}) was $1.26\pm0.07 \times 10^{-14}$ mol·cm^{-2}·s^{-1}and affinity constant ($K_{Tm\text{-}R}$) was $10^{7.04}$ M^{-1}. In the presence of citric acid, malic acid, and nitrilotriacetic acid (NTA), the J_{int} for Tm was more than one order of magnitude higher than that predicted by the BLM. The mechanisms behind the failure of BLM were explored. The possibilities of a piggyback internalization or a limiting mass diffusion were ruled out. The most likely explanation was that the increased uptake appears to be due to the bioaccumulation of a ternary Tm complex.

Effects of water chemistry on the response of invertebrates to rare earth elements (PL)

Jim McGeer[1], Alexandria Loveridge[1], Che Lu[1], Oliver Vukov[1] and D. Scott Smith[1]

[1]Wilfrid Laurier University

The toxicological understanding of rare earth elements (REEs) in the aquatic environment is very limited but there is increasing concern as the use of these elements continues to grow. There are no water quality guidelines or criteria for REEs. The overall objective of this research is to contribute to the understanding of the potential impacts of REEs in aquatic environments. In this particular project, the toxicity-modifying influences of cationic competition (Ca, Mg and Na) and dissolved organic matter (DOM) on the toxicity of Ce, Sm and Dy to *Hyalella azteca* and *Daphnia pulex* was assessed with the goal of developing toxicity prediction models. Standard methods (Environment Canada) were used for culture and testing, which was done in intermediate hardness waters (60 µg·L^{-1} $CaCO_3$, pH 7.2, Ca 0.5 mM, Mg 0.15 mM and 21-23C). The protective effect of cationic competition was tested with Ca (0.1 to 2.0 mM), Mg (0.03 to 0.5 mM) and Na (0.1 to 2.0 mM), and in general Ca mitigated the impact of REEs but Na and Mg did not. As well, DOM reduced toxicity of all the elements tested. The applicability of geochemical equilibrium modelling approaches to understanding site-specific toxicity-modifying factors is developed in the context of the BLM. [This research is funded by Natural Sciences and Engineering Research Council of Canada (NSERC) through a Strategic Grant, in partnership with Environment Canada.]

The effects of water chemistry and organism source on the toxicity of dysprosium to *Hyalella azteca* (PO)

Che Lu[1], D. Scott Smith[2], D. George Dixon[1] and Jim McGeer[2]

[1]University of Waterloo, [2]Wilfrid Laurier University

Rare earth elements (REEs) are a series of seventeen chemical elements containing mostly lanthanides. Due to their multiple applications in industry and consumer electronics, global demand and production of REEs have been increasing. Canada has the potential to be one of the major REE producers and its reserves are mainly located in northern regions. In this project, toxicity tests with *Hyalella azteca* neonates are conducted on the heavy REE dysprosium (Dy). *Hyalella* were collected from different locations across Ontario—Fort Hope, Hannah Lake, Low Water Lake and Daisy Lake—and cultured in the lab. Tests were done following standard methods (EPS 1/RM/33), were 96 hours in duration and were begun with neonates 2- to 9-days-old. A range of exposure concentrations was used, typically 200, 800, 1600, 3200, 6400 $\mu g \cdot L^{-1}$, plus a control in moderately soft water (60 mg $CaCO_3 \cdot L^{-1}$ hardness), pH 7.3 and 23°C. Sample of exposure solutions for characterization were collected before and after the exposure. Results show that *H. azteca* from Fort Hope had sensitivity to Dy similar to those from Hannah Lake, but were more tolerant than those from Low Water Lake and Daisy Lake. Tests at different concentrations of Ca^{2+} or Mg^{2+} or Na demonstrated the protective effect of Ca^{2+}, but not Mg^{2+} and Na against Dy toxicity. Dissolved organic matter (Suwannee River source) also provides a protective effect (at 8 mg $DOC \cdot L^{-1}$). Results are discussed in the context of the biotic ligand model. [This research is funded by Environment Canada with contributions from Avalon Rare Metals Inc., as well as PerkinElmer.]

Effects of water chemistry on the toxicity and bioaccumulation of selenite to *Hyalella azteca* (PO)

Lesley Milne[1], Warren Norwood[2] and D. George Dixon[1]

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Selenium (Se) is an essential non-metal trace element that is sometimes considered a metalloid. It can be toxic when concentrations exceed essential levels. Anthropogenic sources of Se are often by-products from metal ore refining, but Se is also of interest due to its use in manufacturing. Se is more toxic in an organic form and from a dietary route of exposure; however, the inorganic waterborne forms can also have toxic effects. As part of the Canadian government's Chemicals Management Plan, it is of interest to determine how different water chemistry affects mortality, growth, and bioaccumulation in *Hyalella azteca* after acute (7-day) and chronic (28-day) exposures to selenite. These endpoints were examined after exposure to a concentration series of sodium selenite (Na_2SeO_3) at three different levels of pH, dissolved organic carbon (DOC),

or alkalinity in soft water. In acute (7-day) exposures, it was found that a higher pH had a protective effect. However, in chronic exposures (28-day), mortality at pH 8.5 greatly increased. Increasing dissolved organic carbon from a natural source increased toxicity. The lethal body concentration of Se in these high DOC treatments was significantly lower. As well, growth was significantly reduced compared to control. Low alkalinity exposures increased selenite toxicity.

Toxicity of stable strontium in surface freshwaters of Canada (PO)

Laura Pacholski[1]

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Limited data exists on the toxicity of stable strontium to freshwater aquatic organisms. Without sufficient data, it is not possible to establish robust freshwater aquatic guidelines or a chronic effects benchmark to monitor and manage the potential impact of strontium in aquatic freshwater environments. Data from lethal and sublethal toxicity testing on the effect of stable strontium to five aquatic organisms native to Canada resulted in *Pseudokirchneriella subcapitata* three-day IC_{25} 53 $mg \cdot L^{-1}$, *Ceriodaphnia dubia* six-day LC_{50} 206 $mg \cdot L^{-1}$ and IC_{25} 34 $mg \cdot L^{-1}$, *Daphnia magna* 21-day LC_{50} 122 $mg \cdot L^{-1}$ and 21-day IC_{25} 49 $mg \cdot L^{-1}$, *Pimephales promelas* seven-day LC_{50} 354 $mg \cdot L^{-1}$ and seven-day IC_{25} 319 $mg \cdot L^{-1}$, *Oncorhynchus mykiss* 21-day LC_{50} 286 $mg \cdot L^{-1}$. These new data, added to existing literature available, meet the 2007 Canadian Council of Ministers of the Environment requirements for deriving Type B2 water quality guidelines and allow for derivation of a more robust chronic effects benchmark for strontium.

Predicting the chemical and biological effects of tertiary metal mixture (nickel, copper, cadmium) to aquatic plant *Lemna minor* (PO)

Yamini Gopalapillai[1] **and Beverley Hale**[1]

[1]University of Guelph

In nature, contamination of aquatic environments may be a mixture of metals, and this is particularly true of natural waters contaminated by mining effluents, since mineral deposits are commonly an association of multiple metals. For example, nickel mineral deposits mined from Sudbury (Ontario, Canada) are often associated with copper and cadmium. However, water quality guidelines (WQGs) for protection of aquatic life are not designed for multiple contaminants or multiple forms of the same contaminant that co-occur. Rather, WQGs are overwhelmingly based on dose-response studies of single contaminants. Resolving the inability to predict risk from metal mixtures in waters surrounding Canada's many current and legacy extractive mining sites is a high priority for Environment Canada, Health Canada, as well as base metal mining companies. Mixtures of forms of the same metal and mixtures of different metals may have toxicity that would

not be predicted additively. Our study aimed to validate the “concentration addition” approach to predicting the toxicity of a tertiary metal mixture (nickel (Ni), copper (Cu), cadmium (Cd)) to *Lemna minor* (one of Environment Canada’s recommended test plant species for bio-monitoring of mining effluents) using a central composite design (CCD). The CCD is an efficient experimental approach that uses a rotatable incomplete factorial design to model a response surface, without requiring a complete three-level factorial, which is very resource-intensive for conducting toxicity studies. Preliminary results show that toxicity of Ni, Cu, plus Cd is not concentration additive, likely due to competition amongst the metals for uptake into the plant.

Fate and Effects of Pulp and Paper Mill Effluents

Vision2020 Canada Natural Advantage (PL)

Robert Larocque[1]

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Forest products industry had to make difficult decision and embrace transformation following the late 2000's recession. In May 2012, the Forest Products Association of Canada (FPAC) unveiled an ambitious vision for the future of the forest products industry with goals for jobs, growth and continuous improvement on our environmental record. The sector is transforming and now growing again with innovation at the core of its productivity and growth strategy. As a collective, the industry is now moving forward to find the best technological, social, environmental and economic pathways to progress. FPAC would present an overview of the forest products Vision, progress to date, challenges, linkages between transformation and environmental record and potential actions all stakeholders could take to achieve Vision2020 goals.

Restoration of historically contaminated intertidal sites in British Columbia (PL)

Katerina Vassilenko[1], **Vicki Marlatt**[2], **Jeremy Jackson**[3], **Chris Kennedy**[3] **and Shannon Bard**[4]

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Howe Sound is a Pacific Northwest fjord located immediately north of Vancouver, British Columbia. Historically, this site has been impacted by effluents from several industries including two pulp mills, the Britannia copper mine, and an alkali plant. Since the initiation of more stringent enforcement of environmental standards in the 1980s and subsequent modifications in industrial processes for pollution abatement, as well as closure of the alkali plant and a pulp mill, improvements in water and sediment quality and decreases in associated toxicity have been observed. However, recovery of fish populations and intertidal communities has been slow or nearly negligible, particularly at heavily impacted sites. One highly impacted site, Darrell Bay, is located at the northern end of the fjord in close proximity to a decommissioned pulp mill and the former alkali plant. Rocky intertidal habitat at this site was significantly altered many decades ago—likely during wharf construction to service the former pulp mill, which necessitated shoreline bank stabilization. The goal of this study was to (a) characterize present chemical and biological conditions at Darrel Bay and (b) test the efficacy of intertidal habitat restoration methodologies. The number of species present at Darrell Bay

increased from 6 to 16 between 1990 and 2012 (compared to upwards of 94 species at reference sites). In 2012, a first restoration trial was undertaken at the site, when bare rocks were used to create an intertidal reef. After 12 months, the artificial reef was colonized by 9 of the 16 species inhabiting the site. In 2013, rocks seeded with sessile species were brought from a more diverse site in Howe Sound and used to create a new intertidal reef with different architecture. After 8 months, 21 species were observed on the new reef, including motile species. In order to characterize site conditions, sediment quality and its toxicity were examined and three mussel-based biomarkers measured (i.e., scope for growth (SFG), metallothionein (MT) and ethoxyresorufin O-deethylase (EROD) activity). In summary, site characterization and applied restoration methodologies were successful in stimulating intertidal community recovery, which could be implemented at other historically impacted intertidal sites in a multi-stressor environment.

Use of stable isotope analyses to define exposure of white sucker (*Catostomus commersoni*) to pulp mill effluent in Jackfish Bay, Lake Superior (PL)

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Over the last 25 years, the effluent from the bleached kraft pulp mill in Terrace Bay, Ontario, discharging into Jackfish Bay (Lake Superior), has been extensively studied as a Great Lakes Area of Concern. Although the production of dioxins and furans has ended, biologically active chemicals in the effluent continue to reduce gonad size and circulating sex steroid levels in white sucker, *Catostomus commersoni*. White sucker were chosen as a sentinel species due to their abundance, early maturation and benthic foraging nature. White sucker from Jackfish Bay migrate upstream through adjacent water bodies (Tunnel Bay and Jackfish Lake) to reach a suitable habitat to spawn (Sawmill Creek). These partial or non-exposed water bodies contain white sucker that also spawn at the same location. Therefore, it is unknown whether spawning white sucker were exposed to pulp mill effluent. Stable isotope ratios of carbon (δ13C) and nitrogen (δ15N) were explored as indicators of pulp mill effluent. The first objective of this study was to determine if white sucker have a different isotopic signature at Jackfish Bay compared to a reference site. Exposed white sucker had depleted δ13C and enriched δ15N signatures compared to reference fish. The second objective was to determine if white sucker isotopic signatures changed between mill operation and closure. The mill closed down in October 2011, and resumed operation in September 2012. Fish were collected twice a year during 2011-2013. When the mill was operating, white sucker had depleted δ13C and enriched δ15N signatures compared to fish caught while the mill was closed (2012). White sucker had a delayed response to mill closure, exhibiting more enriched δ13C and depleted δ15N signatures during August of 2012 compared to May 2012. The third objective was to determine if white sucker present in adjacent water bodies differed from the exposure site, and their origin could be determined during spawning at the same

location. White sucker were caught in the summer at the exposure site and adjacent sites, and contrasted to those caught during spawning in Sawmill Creek. The fish collected at Jackfish Bay had similar δ13C and δ15N signatures to Tunnel Bay fish, indicating that Tunnel Bay is exposed to effluent, and/or that the range of white sucker includes both Jackfish and Tunnel Bay. Fish caught at non-exposed Jackfish Lake had very depleted δ13C and enriched δ15N signatures compared to Jackfish Bay and Tunnel Bay, as expected for a shallow, warm lake. White sucker caught at Sawmill Creek had a distribution of isotopic signatures similar to those found at Jackfish Bay, Tunnel Bay, and Jackfish Lake, which indicate that the fish caught during spawning come from different regions of exposure. Stable isotopes of carbon and nitrogen can be used as indicators of effluent due to the difference in isotopic signatures of fish between exposed and reference sites, mill operation and closure, and during spawning.

Mill effluent fate and effects for Irving Pulp and Paper (PL)

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J.D. Irving was among the first in Canada to proceed with Identification of Cause (IOC) and Identification of Solution (IOS) for the fish component in the context of the *Pulp and Paper Effluent Regulations'* Environmental Effects Monitoring (EEM) requirements. Recently, two of three mills successfully identified solutions, while a third mill continues to work on IOC/IOS. The uniqueness of the mill processes combined with the various methods of treatment used at each mill have clearly highlighted the fact that one solution does not fit all, and that solutions only come through understanding and embracing the uniqueness of each mill. This presentation will walk the audience through the journey to IOS for one mill in particular. This journey includes detailed toxicity identification evaluation (TIE) work involving chemical characterization combined with lab-scale validation. In the end, a high-level view is taken in order to connect potential effects from a specific fraction of the effluent to preventing it from reaching the receiving environment at environmentally relevant concentrations.

Chemical characterization of a reproductively inhibitory and neuroactive TMP mill effluent and its balsam fir feedstock using UPLC-QTOF (PL)

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Using the *in vitro* glutamic acid decarboxylase (GAD, the key GABA synthesis enzyme in the brain) assay, we performed bioassay-guided fractionations of a thermomechanical pulp (TMP) secondary-treated effluent capable of rapidly inhibiting

reproduction in the 5-day fathead minnow (*Pimephales promelas*) spawning assay. We concentrated 90 L of effluent using reverse-osmosis and then sequentially extracted the resulting 3-L concentrate with hexane and ethylacetate. Solvent and aqueous phases were tested in the GAD assay, with the two ethylacetate extracts inhibiting GAD by 48% and 43% ($p<0.05$). Discriminant analysis in both positive and negative ion mode of active and non-active fractions using principal component analysis (PCA) and S-Plot analysis with MarkerLynx using Metlin and Chemspider databases identified three discriminant metabolites from the active ethylacetate fractions. Co-chromatography with commercially available standards confirmed the presence of 4-acetylpyridine, but when tested in the GAD assay it was inactive. The GAD inhibitors dehydroabietic and abietic acid were observed in one of the inactive hexane extracts.

Balsam fir (*Abies balsamea*) accounted for 75% of the wood feedstock for the studied effluents. We fractionated an ethylacetate extract of balsam fir wood by silica gel chromatography to generate 31 chemically distinct fractions. When tested for GAD activity, 20 of these fractions were inhibitory (6-40% inhibition; $p<0.05$). We created 6 sub-fractions using preparative-scale HPLC of the most active fraction (F20); all of these sub-fractions were active (15-50% GAD inhibition). When we performed another fractionation of the sub-fraction with adequate yield and activity (45% GAD inhibition), activity was again observed in all 6 generated fractions but the activity of the most inhibitory fractions was greatly reduced (12-26% inhibition). Yields were too low and the chemical complexity of samples too high for further sub-fractionations to be performed. We used UPLC-QTOF to analyze an adjacent fraction (F21; 38% inhibition of GAD). We identified (+)-lariciresinol as a major peak in the sample, but it was inactive in the GAD assay. When we compared this active fraction with the active ethylacetate effluent fractions using retention time, elemental composition, and isotopic fit, we were able to identify 16 metabolites common between the effluent and balsam fir active fractions. This suggests that bioactive compounds from feedstock conifer species are able to survive the pulping and effluent treatment processes. The exact nature of the GAD-inhibitory chemicals remains to be determined. These approaches may yield new information on the neuroactive factors in effluents that cause inhibition of spawning in teleosts.

Ovulation but not milt production is inhibited in fathead minnows (*Pimephales promelas*) exposed to a reproductively inhibitory pulp mill effluent (PO)

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A five-day fathead minnow (FHM; *Pimephales promelas*) spawning assay is used by industry to monitor pulp mill effluent quality, with some mill effluents capable of completely inhibiting spawning. The purpose of this presentation is to characterize the effect of an inhibitory effluent on egg and milt production in FHM. Eight tanks were treated with an inhibitory effluent while eight were kept with clean water. Each tank contained two males and four females as per the 5-day FHM spawning assay used by

industry. Females were stripped of ovulated eggs and males of milt in four effluent-exposed and four control tanks. Eggs oviposited in every tank were also counted and checked for fertilization and data analyzed with 2-way ANOVA. We show that female, but not male, fathead minnow reproductive function is impaired in the five-day FHM spawning assay used by industry to evaluate pulp mill effluent quality in Canada. Milt production was not changed in the control or exposed males mid-way and at the end of the five day exposure ($p>0.05$; $n=8$). Total egg production (stripped oviposited) was impaired ($p<0.05$) in fathead minnows exposed to effluent (288 eggs/tank, $n=4$ tanks) compared to those in control tanks (753 eggs/tank, $n=4$ tanks). Our results indicate that males are able to detect female signals and prepare appropriately for spawning while in females inhibition of ovulation is occurring somewhere along the hypothalamus-pituitary-gonad reproductive axis. These results suggest female-specific neuroendocrine disruption and provide mechanistic insight into an assay used by industry to assess pulp mill effluent quality.

Coastal marine biodiversity along contamination gradient in Howe Sound, British Columbia: Historical perspective on pulp mill effluents and other stressors (PO)

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Howe Sound is a Pacific Northwest fjord located immediately north of Vancouver, British Columbia. Historically, this basin has been impacted by industrial activities including pulp mills, a copper mine, a chlor-alkali plant, and log boom storage. In the 1980s, more stringent environmental standards were initiated, leading to modifications in industrial processes and pollution abatement. The basin has also seen the closure and remediation of the chlor-alkali plant and one pulp mill. Since then, improvements in water, sediment quality and contaminated sediment toxicity have been observed. However, ecosystem recovery (e.g., fish populations and intertidal communities) has been slow or nearly negligible, particularly at or near heavily impacted sites. For example, the number of intertidal species increased from 4 to 11 at one of the most impacted sites (Darrell Bay), compared to a baseline 98 species at a reference site (Hornby Island). We propose that factors that may delay the recovery of the ecosystem are historical and/or current sediment contaminants and other stressors, including the degradation of physical habitat availability and quality. A set of historical and current information has been compiled to better understand: (a) effects of complex impacts and stressors on the Howe Sound ecosystem; (b) interactions between ecosystem elements (e.g., subtidal fish community, intertidal community, physical habitat quality and quantity, and current and historical contamination gradient); and (c) impacts from sources (e.g., pulp mill, log boom storage, copper mine). The information collected includes a summary of past industrial

activities, historical and current contamination sources, current environmental quality data from selected sites, historical and current intertidal communities data, and historical fish community data. The constant evolution of impacts and stressors to the Howe Sound basin is discussed, as well as suggested future work that will enable a better understanding of ecosystem functioning. Recognizing and understanding multiple stressors, with a focus on the interplay between biological, chemical, and physical factors, will benefit the management of aquatic contaminated sites, and improve decision making processes.

Municipal Wastewater Effluent Impacts and Remediation

Assessment of the reproductive performance of feminized rainbow darter (*Etheostoma caeruleum*) from the Grand River, Ontario (PL)

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The Grand River in the area of Kitchener-Waterloo, Ontario, Canada has been shown to have a high incidence of intersex in male rainbow darter (*Etheostoma caeruleum*) downstream of wastewater treatment plants (WWTPs). This high rate of intersex is associated with decreases in androgen production and delayed gonad development. What is not clear is whether there is a direct impact of these measures on reproductive success. Does intersex matter? To test this question, fish were collected directly up and downstream of the Kitchener WWTP, and at a reference site upstream of the city limits in three consecutive years. Gametes were stripped from fish and were manually fertilized on site in order to measure fertilization success and embryo survival to hatch. Gonads were then collected and weighed prior to allocation for either sex steroid production or histological analysis. Fertilization success and embryo survival were lower in fish collected from sites near the WWTP. While health measures such as androgen production were suppressed and intersex severity was higher near the WWTP, these health measures did not directly correlate with an individual's ability to successfully produce progeny by artificial fertilization. This suggests that while measures of reproductive health are good predictors of the general populations' fitness, they are not good predictors of an individual's reproductive fitness.

Genotoxicity testing of municipal wastewater: The next step for safeguarding the environment and human health? (PL)

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Federal and provincial governments have regulations for municipal wastewater facilities requiring that effluent not be acutely toxic and that selected parameters meet specified standards. Regulatory requirements can also include the consideration of uses of the receiving environment and development of standards that provide protection for those uses. Acute and chronic toxicity testing, assimilative capacity studies and environmental monitoring can be conducted to develop standards that are protective of

the environment and human health. Biosensors using engineered strains of bacteria can also be applied to evaluate water quality and optimize water treatment technologies. Biosensors can be used to detect the genotoxic and mutagenic effects of chemicals and metabolic by-products from different water treatment methods. Testing using biosensors can be conducted in non-specialized laboratories to provide insight into the biological effects of emerging contaminants such as pharmaceuticals and personal care products in municipal wastewater. Wastewater treatment facilities across Canada are being upgraded but the full spectrum of biological effects of municipal wastewater is not currently being considered. Wastewater treatment methods and site-specific water chemistry are known to affect the genotoxicity of treated water. Many emerging contaminants, as well as disinfection by-products in wastewater effluent are known to be genotoxic. Genotoxic compounds in wastewater effluent can affect not only the genetics of aquatic ecosystems, but also human health in communities that depend on surface water for drinking water. Testing wastewater effluent for genotoxicity and mutagenicity can improve the evaluation of treatment methods, reduce the release of genotoxins and mutagens to the environment, and help protect the environment and human health for generations to come.

Operational efficiency of an integrated constructed wetland system for treating domestic wastewater in South East Queensland, Australia (PL)

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Population growth, industrialization, climate uncertainty and agricultural operations are putting increasingly greater demands on freshwater supplies, and thus water recycling is being widely explored as a way to alleviate the burden on freshwater resources. Strategies for water recycling are of particular interest in Australia, where diminishing freshwater resources and drought are common concerns. Municipal wastewater represents one of the largest sources of water that can be considered for recycling. However, in order to be widely implementable, water treatment options must be operationally simple, cost-effective, and produce water that is of a quality fit for reuse (i.e., protective of both environmental and human health). As such, a comprehensive evaluation of an integrated constructed wetland wastewater treatment system in South East Queensland was performed, to determine the capacity for this “natural” treatment option to remove nutrients, pathogenic microorganisms, chemical contaminants, and *in vitro* and *in vivo* toxicological potential. The system proved to be highly effective at removing nutrients and microbes. Many chemicals were poorly removed, but influent and effluent concentrations fall below public health regulatory standards and *in vitro* and *in vivo* tests demonstrate reduced toxicological potential in final effluent.

Simulation of the fate of selected pharmaceuticals and personal care products in a highly impacted reach of the Grand River Watershed in southern Ontario (PL)

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A wide variety of trace organic contaminants are present in municipal wastewater treatment plant (WWTP) effluent. There is also a diversity of potential mechanisms in effluents that can impact the biological function in aquatic organisms. However, WWTP effluent is a complex mixture and assessing the linkages and severity of the effects associated with trace organic contaminants can be difficult. This study describes a water quality modelling exercise completed to: (a) determine the fundamental mechanisms of trace organic contaminant fate and transport; and (b) predict their concentration in a highly impacted reach of the Grand River Watershed in southern Ontario. Fate and transport of triclosan (antiandrogenic personal care product) and selected pharmaceuticals (venlafaxine, naproxen, and carbamazepine) were modeled using the calibrated WASP 7.5 model. The simulation revealed that the flow-driven transport processes such as advection and dispersion greatly affect the behaviour of the target compounds in the aquatic environment. The fate mechanisms such as biodegradation and photolysis can also play a role in the dissipation of these compounds in surface water. The water quality model can now be applied to a number of future applications such as the assessment of the WWTP infrastructure upgrades and the prediction of fate and transport of other tracer organic contaminants within the watershed boundaries.

The contribution of pharmaceutically active compounds from healthcare facilities to a receiving sewage treatment plant in Ontario, Canada (PL)

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The presence of pharmaceutically-active compounds (PhACs) and other emerging contaminants released from healthcare facilities (2 hospitals and a long-term-care facility) to a sewage treatment plant (STP) in a large urban watershed was evaluated. Effluents from an additional hospital and veterinary clinic outside the watershed were also monitored for comparison purposes. We detected 14 of the 24 steroids and hormones and 88 of the 117 PhACs in the analytical suite at least once. Commonly used substances such as cotinine, caffeine and its metabolite1, 7-dimethylxanthine, the analgesics ibuprofen and naproxen, the antidepressant venlafaxine and DEET (insect repellant) were detected in samples from all the sites monitored. Concentrations detected in the large specialty hospital outside the watershed were similar to those within the watershed. Cytotoxic drugs (tamoxifen and cyclophosphamide) and compounds used to visually

enhance x-rays (iopamidol and diatrizoic acid) were occasionally detected in hospital effluents. Analysis for antibiotics indicated that azithromycin, clarithromycin, ciprofloxacin, erythromycin, ofloxacin and sulfamethoxazole were consistently detected in hospital wastewaters in and outside the watershed as was the common antibacterial agent including triclosan. Fifteen compounds contributed greater than 1% of the PhACs load to the receiving STP from the healthcare facilities monitored (acetaminophen, albuterol, amphetamine, ciprofloxacin, codeine, diatrizoic acid, diphenylhydramine, furosemide, noverapamil, ofloxacin, oxycodone, sertraline, sulfamethoxazole, triclosan and trimethoprim). This survey demonstrates that point source discharges from healthcare facilities make a small contribution to the overall pharmaceutical load when compared to the total concentration entering a STP; however, this may vary with the compound and the size of the receiving treatment plant and watershed.

Does pharmaceutical exposure mediate risks associated with exposure to heavy metals? An epidemiological investigation (PL)

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Pharmaceuticals and personal care products (PPCPs) are emerging aquatic contaminants of concern. Prescription drug use is prevalent and increasing in the developed world, and abuse of prescription drugs is a worldwide concern. While prescription drugs are intended to improve health, we are now realizing that a myriad of unintended health consequences may occur. One potential unintended consequence, which has received limited attention, is the effect of prescription drug use on the risks associated with exposure to more common environmental contaminants such as toxic heavy metals. Here, the objective was to understand if human pharmaceutical use can be associated with alterations in biomarkers of exposure to common toxic heavy metals (total blood mercury [Hg], total blood lead [Pb], and total blood cadmium [Cd]). Prescription drug use and metals biomarker data were obtained from the U.S. National Health and Nutrition Examination Survey (NHANES) (n=7133). Preliminary analyses indicate that individuals taking anti-infective agents had 20-54% lower Hg, 14-42% lower Pb, and 19-30% lower Cd than those taking a variety of other prescription drug types. Within the anti-infective category, it was notable that cephalosporins and tetracyclines had an opposite relationship for Hg and Pb; cephalosporins were associated with lower Hg than tetracyclines, while tetracyclines were associated with lower Pb than cephalosporins. More detailed statistical analyses of the NHANES data are in progress and will be presented. These results suggest that exposure to PPCPs commonly utilized by society may mediate exposures to other contaminants, such as toxic heavy metals. Future work is needed to better resolve the underlying mechanisms and determine the hazards associated with such real-world exposures to chemical mixtures.

Assessment of the estrogenic potential of wastewater treatment plant effluent in the Grand River, Ontario (PL)

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The Grand River in southern Ontario, Canada, is subject to various anthropogenic impacts, including contaminant inputs from 30 wastewater treatment plants. Some of the substances detected in these effluents can mimic naturally occurring hormones and therefore have the potential to interact with the endocrine system of fish. Contaminant interaction with receptors (molecular initiating event) can induce responses at higher levels of biological organization that manifest in changes such as sex steroid production, decreased gonad size and intersex condition. Previous studies conducted in the Grand River have demonstrated a variety of effects in fish downstream of the wastewater outfalls, including a significant increase in intersex. However, preliminary studies have not indicated a major increase in vitellogenin (VTG), a biomarker often associated with estrogen exposure. The objective of this study was to assess the estrogenic potency of the effluents in the central Grand River and to determine whether VTG induction would occur in response to effluent exposure by production of either plasma VTG or mRNA.

For this purpose we caged three species of fish: rainbow trout (RBT; *Oncorhynchus mykiss*), fathead minnow (FHM; *Pimephales promelas*) and rainbow darter (RBD; *Etheostoma caeruleum*) for 14 days at two sites upstream and three sites downstream of the wastewater discharge. A subset of caged fish were injected with 17α-ethynylestradiol (EE2), a potent estrogen at 10 $mg \cdot kg^{-1}$ of wet-weight, to evaluate and compare the species responsiveness in terms of VTG induction to a known estrogen. Plasma was collected from RBT and FHM for analysis of VTG by enzyme-linked immunosorbent assay (ELISA), and liver tissue was collected from RBD for polymerase chain reaction (PCR) analysis of VTG mRNA. Total estrogenicity was characterized using the yeast estrogenic screening (YES) assay. Extracts were also used in an effect-directed assessment (EDA) to isolate specific estrogenic chemicals. The results of combined EDA with YES showed that the total estrogenicity of the effluent exceeded 1 $ng \cdot L^{-1}$ E2eq (estradiol equivalents), which could explain the absence of VTG induction in the exposed RBT and FHM at all five sites. On the other hand, the results of EE2-injected fish (RBT and FHM) indicated that there was some sort of suppression in VTG production in those fish exposed at the upstream site. In terms of species-specific responses we noticed similar patterns in both RBT and FHM which also showed time-dependent increase in VTG production. VTG mRNA analyses performed on RBD liver samples are currently in progress. The outcome of all these analyses will provide information that will help us better understand species-specific and site-specific responses in fish exposed to municipal wastewater effluents.

Derivation of a Canadian Water Quality Guideline for the protection of aquatic life: Carbamazepine (PL)

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Carbamazepine (CBZ) is a drug commonly prescribed as an antiepileptic, and also used for the treatment of other conditions (e.g., pain associated with trigeminal neuralgia, mania). The annual quantity of CBZ used in Canada is approximately 21,450 kg per year. The vast majority (>20,000 kg per year) is used in private homes, and the remainder is used in hospital. The main route of entry of carbamazepine into the aquatic environment is via municipal sewage treatment plant effluent. Other possible sources are combined sewer overflows during rain events, leakage from sewage systems, septic systems, and biosolids applied to agricultural land. Carbamazepine has been found to be recalcitrant to many conventional wastewater treatment processes, and as a result has been detected globally in municipal sewage treatment plant effluent, surface water, groundwater, and treated drinking water. Given its ubiquity, and the fact that carbamazepine is designed to be bioactive, it is of concern for aquatic life. A Canadian Water Quality Guideline for the protection of aquatic life for carbamazepine has been developed, and will be discussed.

Coupling *in vitro* and *in vivo* neurochemical-based assessments of wastewater effluents from the Maumee River Area of Concern (PL)

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In this study we utilize *in vivo* and *in vitro* approaches to study whether real world effluents released in the Maumee River (Toledo, OH) Area of Concern (AOC) contain neuroactive substances that may impair fish reproduction and behaviour. Our approaches help extend the concept of endocrine disruption beyond routine bioassays (ER, AR, TH) under the premise that toxicants may also interact with and disrupt the function of neurotransmitter receptors and enzymes that play critical roles in vertebrate reproduction and behaviour. Cell-free methods were used to study such interactions and to compare the *in vivo* and *in vitro* responses. First, 288 fish (*Pimphales promelas*) were exposed in cages to river water at 8 different sites along the Maumee River, including several in close proximity to wastewater treatment plant (WWTP) discharges. After four days of *in situ* exposure, brains were sampled and analyzed for N-methyl-D-aspartate (NMDA) receptor binding, and monoamine oxidase (MAO) and glutamine synthetase enzyme activity. The preliminary work shows that fish caged downstream of a major WWTP had increased MAO activity (66% and 35% in males and females respectively), and

that NMDA receptor binding was also significantly changed (30% decrease in females). Second, *in vitro* studies were performed on river water extracts (final concentration 5x) to see if they interfere with the aforementioned neurochemicals studied *in vivo*. The *in vitro* results suggest that extracts optimized for recovery of alkylphenols significantly impacted the NMDA receptor binding (15% decrease), whereas those optimized for recovery of steroid hormones induced binding (up to 34%) in fish. In summary, our work thus far suggests that wastewater effluents discharged to the Maumee River AOC contain chemicals that may directly interact (and possibly interfere) with neurochemicals that are important in fish reproduction and behaviour. In addition, the work here (via a U.S. Environmental Protection Agency Science to Achieve Results (STAR) grant) is taking next steps to identify key neurochemical indicators, resolve *in vitro* and *in vivo* responses, and compare responses across taxa.

Prozac disrupts multiple physiological systems in teleost fish (PL)

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The therapeutic ingredient in the antidepressant Prozac, fluoxetine (FLX), and other selective serotonin reuptake inhibitors (SSRIs) serve as compelling examples of environmental pharmaceuticals of concern. Several SSRIs and metabolites have been detected in wild fish, indicating the potential to bioconcentrate. There are now a range of clear effects of environmentally-relevant FLX levels (range: 12-540 $ng \cdot L^{-1}$) on behaviour, physiology, neuroendocrine function, reproduction, feeding and growth in several teleost species. The total load of all measured SSRIs may reach 3.2 $\mu g \cdot L^{-1}$ near sewage outfalls, with levels decreasing downstream. We have shown that environmentally-relevant levels of FLX alter the expression of neuroendocrine genes in the goldfish hypothalamus that are important for the control of spawning and feeding. Other data indicate upsets in hepatic metabolic processes in goldfish (*Carassius auratus auratus*) and zebrafish (*Danio rerio*). Physiological experiments indicate that such changes at the level of the brain are linked to altered blood sex hormone levels, reduced food intake and weight loss in several teleost species. Sexually mature male goldfish were exposed up to 2 weeks to FLX in the water (540 $ng \cdot L^{-1}$). Following this, males were treated with a pulse of female primer pheromone 17,20-dihydroxy-4-pregnene-3-one or the releaser pheromone, prostaglandin F2, and sperm release and neuroendocrine responses assessed. Both basal and pheromone-stimulated milt production were suppressed by pre-exposure to FLX. Published data on zebrafish also indicate that waterborne FLX suppresses ovulation. Together, these data in teleost fish indicate that FLX can suppress both female and male reproductive function. In contrast, little is known about the potential anti-reproductive or anti-feeding effects of SSRIs on amphibians, aquatic insects or crustaceans downstream of sewage treatment outflows. Given that serotonin is important in multiple physiological control systems in both vertebrates and invertebrates, it will be important to examine the effects of SSRIs in

these other species. This presentation will end with a proposal to rigorously test the hypothesis that environmental FLX exposure leads to deleterious effects on natural aquatic populations. [Supported by Natural Sciences and Engineering Research Council (NSERC) and the University of Ottawa Research Chair Program.]

Quantification of polycyclic musks HHCB and AHTN in fathead minnows from Edmonton, North Saskatchewan River, around Gold Bar wastewater treatment plant (PL)

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Synthetic musks are fragrances added to personal care products, including perfumes, detergents and other household cleaning products, to make them fragrant. They are eventually released to rivers and lakes, and because they are designed to resist degradation, they are persistent in the environment.. Previous studies showed musks to have sublethal effects and endocrine disruption properties. The current study examines two synthetic products of the polycyclic musk family: Galaxolide, or 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta(g)-2-benzopyrane (HHCB), and Tonalide, or 7-acetyl-1,1,3,4,4,6-hexamethyltetrahydronaphtalene (AHTN), which are the most extensively used musks in the industry. The chemical properties of these two contaminants result in their detection in several environmental compartments, including water, fish, sediments, and air. The bioaccumulation potential of these musks was examined in fathead minnows (*Pimephales promelas*) that were collected at six different sites near the Gold Bar wastewater treatment plant, which releases its effluents into the North Saskatchewan River in Edmonton, Alberta. Preliminary results indicate that these musks are widely detected in fish near these wastewater effluents. The concentrations of HHCB and AHTN in fish that were collected in the plume of the treated wastewater discharge ranged from 1.99 to 4.96 $\mu g \cdot g^{-1}$ of fish and from 39.36 to 292.80 $ng \cdot g^{-1}$ of fish, respectively.

Identification of the pathways that mediate the actions of environmental pharmaceuticals on spawning success in the zebrafish (PL)

Glen Van Der Kraak[1], Madelyne Cosme[1] and Andrea Lister[2]

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We routinely use zebrafish (*Danio rerio*) as a laboratory model to assess the impacts of effluents from municipal water treatment plans on spawning success. We were particularly interested in evaluating whether it would be possible to identify the reproductive pathways affected by constituents that may be present in municipal wastewater effluents by measuring the levels of hormones via enzyme immunoassay, the

expression of specific genes using q-PCR, and ovarian follicular recruitment using histology. In these studies we focused on the actions of an estrogen receptor agonist, ethinylestradiol (EE2), and a phospholipase A2 inhibitor, quinacrine, which interferes with prostaglandin synthesis. Specifically, we looked for effects on spawning success and the pathways controlling follicular recruitment to the ovulatory pool, steroidogenesis, prostaglandin synthesis, oocyte maturation and ovulation. Our studies showed that both EE2 and quinacrine inhibit spawning but do so through different mechanisms. EE2 had multiple actions, including effects on follicular recruitment (reduced ovarian size and reduction in the proportion of cortical alveolus, vitellogenic and mature ovarian follicles), steroidogenesis (reduced luteinizing hormone receptor and aromatase expression) and ovulation (reduced expression of the cytosolic phospholipase A2 and the nuclear progesterone receptor). In contrast, quinacirine had more restricted action targeting oocyte maturation via a reduction in the expression of the steroid 17α, 20β dihydroxy-4-prenen-3-one and expression of the prostaglandin-metabolizing enzyme cyclooxygenase-2. Collectively, these studies suggest that a targeted assessment of selected endpoints in the zebrafish can be used effectively to identify the mechanisms by which environmental pharmaceuticals can impact reproductive fitness.

Effect directed analysis: A novel approach for the assessment of complex mixtures of contaminants (PL)

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In recent decades, national and international governments have established approaches and regulatory frameworks to assess the risks posed by endocrine-disrupting chemicals (EDCs), compounds that interfere with the hormonal (endocrine) systems of vertebrates. Despite Canada's efforts towards establishing standard test strategies and decision-making criteria for monitoring and assessing sources of EDCs to the environment, there are two key challenges that are hampering the efforts of environmental toxicologists and risk assessors to address this issue objectively and efficiently: (a) the identification and assessment of the toxicologically relevant compounds in complex mixtures; and (b) the biological relevance of the phenomenon of endocrine disruption, and thus EDCs. This presentation will focus on the former question, and will introduce an emerging approach to address current needs for monitoring and risk assessment associated with emerging contaminants in complex matrices such as municipal, industrial or hospital effluents. Early on, it became obvious that the sole use of classic chemical-analytical techniques is not suitable for identifying the causative agents in such complex samples. Specifically, analysis of the vast number of chemicals present in such samples would not only be prohibitively expensive, but simply impossible due to limits in the available analytical methodologies for many chemicals, especially given that often no *a priori* knowledge of the chemicals present in the sample exists. Therefore, a recent approach focuses on integrating chemical- and bio-analytical techniques, making

use of the specific properties of types or groups of chemicals to interfere with specific biological processes. This type of analysis has been coined “effect directed analysis” (EDA), and is based on a combination of fractionation procedures, bio-testing, and subsequent chemical analyses. This paper will introduce the concept of EDA and review case studies to demonstrate the applicability of this approach as an aid in the risk assessment of emerging contaminants in complex environmental samples.

Bioassay-directed characterization of endocrine-disrupting potencies of municipal effluents in Canada (PO)

Tabata Bagatim[1], Sara Hanson[1], Kean Steeves[1], Steve Wiseman[1], Natacha Hogan[1], Alice Hontela[2], Paul Jones[1], John Giesy[1] and Markus Hecker[1]

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In recent years, there has been increasing concern regarding the contribution of municipal wastewater effluents (MWWEs) to the exposure with endocrine disrupting chemicals (EDCs) in surface waters. Conventional treatment technologies are frequently incomplete or inefficient at removing pharmaceuticals and personal care products, as well as other toxins and naturally occurring hormones with endocrine disrupting properties, from wastewater. The potential for these chemicals to adversely affect wildlife and human health has led to national and international regulatory bodies establishing new policies and frameworks to better characterize and monitor the risks posed by EDCs. Although Canada has initiated first steps with the aim of establishing standardized testing and monitoring criteria for EDCs in the environment, our understanding of the contribution of effluents from wastewater treatment plants (WWTPs) to environmental endocrine disruption in Canadian surface waters is incomplete at best. Therefore, the aim of this project is to investigate the effectiveness and efficiency of Canadian WWTPs with regard to removing EDCs from MWWEs. Specifically, influents and effluents from six WWTPs in Saskatchewan, Ontario and Quebec will be analyzed for the presence of (anti-) estrogenic, (anti-) androgenic, and steroidogenesis-disrupting properties using an *in vitro* test battery. Endocrine potentials will be assessed using the T47D-KBluc ((anti-) estrogenicity), MDAkb2 ((anti-) androgenicity), and H295R Steroidogenesis Assay (steroidogenesis disruption). The detection of specific biological *in vitro* potentials will inform targeted chemical analysis of suspected EDCs with the aim of pinpointing causative agents in wastewater. Samples were/will be collected during winter, spring, summer and fall in 2014 and 2015 to investigate the influence of climatic conditions on removal efficiency of EDCs from wastewater. Through the proposed bioassay-directed analysis, this project will characterize the efficiency of current WWTP technologies across Canada to eliminate emerging contaminants of concern, and thus will inform future needs to develop more efficient, economical and effective approaches for removing EDCs from MWWEs.

The removal of biological activity using UV/TiO_2 photocatalysis (PO)

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Advanced oxidation processes (AOPs) are used in treatment technologies to transform and mineralize trace organic compounds. Among the AOPs available, the use of UV irradiation and the photocatalyst, titanium dioxide (UV/TiO_2), has been well received in water treatment studies due to its low cost and high potential to remove persistent trace organic contaminants. However, issues such as the formation of harmful transformation by-products have been suggested. In this study, biological assessments were used to screen the biological activities of target compounds using UV/TiO_2 treatment. The yeast-estrogen screen (YES) bioassay was used to test the estrogenic activity (i.e., potential to disrupt estrogenic function) during the photocatalytic degradation of 17α-ethinylestradiol (EE2), a highly estrogenic component of birth control pills that is known to have biological impacts at very low concentrations. The photocatalytic degradation of EE2 in de-ionized water was completed in triplicates using the commercially available TiO_2 nanopowder (P25). Chemical analysis for EE2 was completed using an Agilent 1200 high-performance liquid chromatography coupled with Agilent 6460 Triple Quadrupole operated under dynamic multiple reaction monitoring. The degradation of EE2 was first-order with an average kinetic rate of 0.0612 per minute. The removal of estrogenic activity was also seen at a rate similar to that of the chemical removal (average rate of 0.0556 per minute). The results imply that there is relatively rapid chemical and biological activity removal of EE2 using P25. Furthermore, the results show that EE2 photocatalysis does not introduce any endocrine active degradation products. This also suggests that the UV/TiO_2 oxidation process is a promising tool in removing endocrine active compounds from water. The removal of target chemicals and biological activity (additional endpoints) using different types of nanoparticles such as TiO_2 nanobelts and TiO_2 powder using Sol-Gel preparation are currently being investigated.

Assessing the potential endocrine disrupting effects of municipal wastewater effluents on fathead minnow (*Pimephales promelas*) populations in Wascana Creek and the South Saskatchewan River, Saskatchewan, Canada (PO)

Sara Hanson[1], Tabata Bagatim[1], Kean Steeves[1], Steve Wiseman[1], Natacha Hogan[1], Alice Hontela[2], Paul Jones[1], John Giesy[1] and Markus Hecker[1]

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The province of Saskatchewan is experiencing a dramatic increase in population growth, resulting in a greater release of municipal wastewater effluents (MWWEs) into

local water bodies. There is concern regarding the impact of contaminants in this effluent, particularly endocrine disrupting compounds (EDCs). This is because conventional wastewater treatment technologies are often incomplete or inefficient at removing pharmaceuticals and personal care products (PCPPs), naturally occurring hormones, and other toxins and contaminants with endocrine-disrupting capabilities from the wastewater. Many of these contaminants have been shown to have effects on growth, development and reproduction of aquatic organisms. There is particular concern about water bodies in the southern prairies, which may be at a greater risk of contamination from EDCs because the receiving water bodies are often small and can consist of up to 100% effluent during dry seasons. Therefore, the aim of this project was to assess the potential endocrine effects of MWWEs on wild fathead minnow (*Pimephales promelas*) populations downstream of two WWTPs in Saskatchewan, namely in Wascana Creek, an effluent-dominated stream in the City of Regina, and the South Saskatchewan River in the vicinity of the City of Saskatoon. Fish were collected up- and downstream of the wastewater treatment plants in early summer and fall during 2014 to investigate potential impacts at several stages of the reproductive cycle. The impact of municipal wastewater on overall fish health, focusing on reproductive effects, was evaluated through histopathology, secondary sexual characteristics, and ovosomatic indices as well as sex ratios and biochemical and molecular biomarkers. Successful completion of this study will help in characterizing the potential endocrine effects occurring in local fish populations exposed to MWWEs in Southern Saskatchewan, and thus will inform future needs to develop more efficient and effective approaches for eliminating EDCs from MWWEs.

Multi-generational exposure of Florida flagfish (*Jordanella floridae*) to select pharmaceutical mixtures (PO)

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Estradiol and non-steroidal anti-inflammatory drugs are some of the most frequently detected pharmaceuticals in the environment because of easy access and high level of use (Santos et al., 2010). More knowledge of the impacts of environmentally relevant concentrations of pharmaceutical mixtures on fish reproduction over multiple generations is required. A study using Florida flagfish (*Jordanella floridae*) breeding harems was conducted to monitor the multi-generational reproductive effects of exposure to 17α-ethynylestradiol (0.1-10 $ng \cdot L^{-1}$), ibuprofen (0.1-10 $\mu g \cdot L^{-1}$), and naproxen (0.1-10 $\mu g \cdot L^{-1}$) in mixtures. Flagfish were placed into breeding harems with 2 males and 4 females per treatment in triplicate alongside controls. The experiment was conducted in 70 L aquaria. The parental generation underwent a pre-exposure and exposure phase, and F1 larval offspring were collected and reared to adults with continual exposure to the pharmaceutical mixtures. Reproductive endpoints of fertilization, hatchability and egg production were of particular interest. Results will be presented pending analysis. While

reproductive effects are well known for many of the pharmaceuticals alone, much less work has been completed on mixtures, and very few studies have observed the multi-generational effects of such mixtures.

Spatial and temporal variability in somatic indices of the rainbow darter (*Etheostoma caeruleum*) in a highly impacted watershed (PO)

Keegan Hicks[1], Meghan Fuzzen[1], Gérald Tétreault[2], Mark McMaster[2] and Mark Servos[1]

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Fish health is commonly assessed as a function of energy allocation, energy storage, and survival. The most practical means of measuring these parameters is to use fish somatic indices, which include relative liver weight and condition (energy storage), relative gonad weight (energy allocation) and age structure (survival). These somatic indices are effective at indicating efficiency, availability, and quality of food resources in addition to metabolic disruption. Somatic indices have been applied in many biomonitoring programs, including Canada's Environmental Effects Monitoring programs, where they have been demonstrated in some fish species to be successful indicators of change in whole populations. The effectiveness of somatic indices in biomonitoring programs will depend on the species, their sensitivity, and the inherent natural seasonal and annual variability in food supply and habitat quality (flow and temperature). This study assessed the ability of somatic indices in the sentinel species rainbow darter (*Etheostoma caeruleum*) to detect site-specific changes resulting from exposure to two municipal wastewater treatment plant (WWTP; Kitchener and Waterloo) effluents over multiple years (2007, 2010-2013). In addition to annual variability, spatial variability was assessed at the watershed scale in the fall of 2012 and 2013. Results indicate that there is more variability among years than there is among sites within years. Condition factor in males and females is consistently increased downstream of the Kitchener WWTP across the years, while relative liver weight and relative gonad weight are more variable. Further analysis will reveal whether natural variability can be discriminated from potential effects of municipal WWTPs. The outcome of this multi-year study is an assessment of the usefulness and relevance of somatic indices in detecting change in rainbow darter populations, and their potential for use in biomonitoring programs specific to wastewater and more generally for watershed scale cumulative effects assessment.

Assessing biological effects of municipal wastewater effluent using the fathead minnow reproductive bioassay (PO)

Kean Steeves[1], Sara Hanson[1], Tabata Bagatim[1], Steve Wiseman[1], Paul Jones[1], John Giesy[1], Alice Hontela[2], Natacha Hogan[1] and Markus Hecker[1]

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The potential impact of municipal wastewater effluents (MWWE) on aquatic ecosystems is one of the growing environmental concerns in Saskatchewan. The growth of many cities in the province has led to an increased amount of wastewater that must be processed, placing greater demands on current capacities of wastewater treatment plants. Many of the receiving aquatic systems are small, and at times of low flow, the make-up of the water can be 100% discharged effluent. In addition to biological waste, MWWE contains a number of emerging contaminants such as pharmaceutical and personal care products (PCPP), and wastewater treatment facilities are often not equipped with technology that can successfully remove these contaminants from the wastewater. This has the potential to affect the local aquatic organisms. In this context, there is particular concern with regard to contaminants that are considered endocrine disrupting compounds (EDCs). EDCs can adversely affect growth, reproduction, and survival of aquatic organisms, which can result in alterations to populations living in the receiving environments. The goal of this project was to determine, using the U.S. Environmental Protection Agency short-term fish reproductive bioassay assay, the effects of MWWEs on the endocrine system of hatchery-raised fathead minnow (*Pimephales promelas*) exposed in the laboratory to effluents collected from both the Regina and Saskatoon wastewater treatment plants. The endocrine disrupting effects were assessed using various reproductive measurements (number of eggs, fertilization success), as well as morphological, histopathological, and molecular/biochemical indicators in both male and female fish. This study assessed the endocrine disrupting potential of the MWWE discharged into Saskatchewan aquatic systems and provided critical information regarding the particular toxicological risks associated with ineffective removal of EDCs from MWWEs. [Funded by the Canadian Water Network.]

Impacts of upgrades to the Kitchener wastewater treatment plant on the distribution and fate of emerging contaminants in the Grand River watershed (PO)

Leslie Bragg[1], Maricor Arlos[1], Emily McCann[1], Patricija Marjan[1] and Mark Servos[1]

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The Kitchener Wastewater Treatment Plant (WWTP) is a conventional activated sludge plant that is currently undergoing major upgrades to improve effluent quality. The upgrades include improving the aeration systems, UV disinfection and decommissioning

the biosolids storage lagoons. Regular sampling and analysis of the effluent for a variety of emerging contaminants has occurred since 2010 and has continued monthly over the last 18 months during implementation of major process changes. There has been a major decrease in total ammonia and an increase in nitrate from the plant, both of which correspond to the upgrades (i.e., nitrification) coming online at the plant. The contaminant profiles, including selected pharmaceuticals, personal care products and estrogens, are contrasted over time and effluent quality. In addition, profiles in several other treatment plant effluents in the watershed with differing processes are compared. Concentrations of selected pharmaceuticals, such as ibuprofen, are greatly reduced with increased treatment, while other contaminants such as carbamazepine are not removed. The estrogenicity of the effluent (measured using yeast estrogen screen as total estradiol equivalents (EEQ)) has dropped significantly from >17 $ng \cdot L^{-1}$ total EEQ to <1 $ng \cdot L^{-1}$. These improvements have potential significance for the environment where significant endocrine disruption has been documented, including high expression of intersex in fish.

Using water quality to measure the success of remedial actions in the St. Marys River Area of Concern (PO)

Corrina Barrett[1] **and Carrie Ginou**[1]

[1]Algoma University

The St. Marys River is a freshwater ecosystem which flows from Lake Superior to Lake Huron and separates the twin cities of Sault Ste. Marie, Ontario (Canada) and Sault Ste. Marie, Michigan (United States). Since the early 1900s, the river has received industrial and municipal wastewater. These inputs have contributed to the deterioration of water quality in the river and have led to the designation of the St. Marys River as an Area of Concern (AOC). Since being identified as an AOC under the Canada-United States Great Lakes Water Quality Agreement, Remedial Action Plans have been developed and implemented on both sides of the bi-national river. The goal of the current research is to monitor water quality in the Canadian portion of the AOC with the purpose of assessing the success of remedial actions, particularly those designed to decrease nutrient inputs from both municipal and industrial sources. Water was grab-sampled from five sites along the St. Marys River and chemical and physical properties were analyzed in the laboratory. Qualitative observations of trophic status were also collected at each site. Preliminary results of this three-year study (2013-2016) show levels of ammonia, nitrogen, phosphorus, chlorophyll a, dissolved oxygen and turbidity consistent with water quality guidelines and generally lower than levels that initially contributed to water quality impairment. Further study will include an additional season of bi-monthly water quality sampling and application of the results to a long-term monitoring plan for the St. Marys River.

Nanotoxicology: Molecular Mechanisms to Ecosystem Impacts

Silver uptake by liposomes used as a model membrane (PL)

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Silver nanoparticles are being increasingly used in industry as they present useful antibacterial properties and affordable production costs. It is recognized that these materials tend to dissolve over time and that the released silver ions could, in principle, lead to toxic effects in the receiving waters. It has also been postulated that the nanoparticles themselves may exert toxicity, but evidence for such direct toxicity remains equivocal. In this project, we are interested in comparing the uptake of dissolved silver and silver nanoparticles by phosphatidylcholine liposomes, this phospholipid being a major component of biological membranes. These vesicles have long been used as a model for biological membranes, as they allow one to study the passive movement of solutes, such as metals, across the lipid bilayer into the liposome inner medium. The liposomes are synthesised in a pH 6 buffer using extrusion techniques. Size exclusion chromatography is then used to transfer the vesicles into another buffer solution and allow determination of potential membrane leakage. The liposomes can then be used for silver uptake studies. The Ag-exposed liposomes are separated from the exposure solution by passing the vesicles through a second size-exclusion chromatographic column equilibrated with a buffer containing a strong silver-binding ligand to remove silver bound to the surface of liposomes. The kinetics of Ag uptake were studied by exposing the liposomes to free Ag or to various silver complexes ($AgS_2O_3^-$, Ag-thiosalicylic acid, Ag-cysteine, Ag-glutathione, and Ag-phytochelatin); uptake was followed over time, and was stopped at various time intervals by separating the liposomes from the silver source. The experimental design and preliminary results will be presented.

Responses of natural bacterial communities at different AgNP exposure frequency (PL)

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Silver nanoparticles (AgNP) are used in a variety of applications for their antimicrobial properties, such as for the prevention of unpleasant odors caused by bacteria in sportswear. Because of their widespread use, their occurrence in aquatic environments could increase. What are the potential impacts on bacterial communities? This study aims to evaluate the response of bacterial communities at different exposure regimes (i.e., frequency and intensity) to AgNP. In 20-L microcosms, bacteria from the St. Lawrence River were exposed to AgNP-citrate (11 nm) once at 1000 $\mu g \cdot L^{-1}$ (Treatment 1), twice at 500 $\mu g \cdot L^{-1}$ (Treatment 2) and 5 times at 200 $\mu g \cdot L^{-1}$ (Treatment 3). After five days, all microcosms received the same amount of AgNP (i.e., 1000 $\mu g \cdot L^{-1}$). Diversity, richness and composition of bacterial communities were analyzed by 454-pyrosequencing after 4, 7 and 18 days of exposure. The results indicated that the same bacterial abundance between treatment and control AgNPs masks a significant change in the diversity and composition of these communities. The changes in the diversity and composition are mainly reflected by an increase of bacteroidetes and a decrease of actinobacteria. Furthermore, an increase of operational taxonomic units (OTUs) for AgNP treatments was observed, suggesting a stimulation of the bacterial diversity. All these changes in the bacterial structure were maintained 18 days after exposure to AgNP, suggesting the lack of resilience of these communities after exposure to AgNP.

Cadmium bioavailability for *Daphnia magna* in presence of single-walled carbon nanotubes (PL)

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The Canadian forestry industry experienced an economic crisis which has led to the development of new forestry products: nanomaterials, including single-walled carbon nanotubes (SWNT). These possess the most notable features among carbon materials and are closely studied for an increasing number of important applications, including water treatment and drug targeting. SWNT have a large specific surface area, giving them high adsorption capacity. This property, very interesting for the transport of molecules, is studied for various applications including the removal of contaminants in wastewater. Increased production of nanomaterials coupled with their applicationto water treatment

raises the question of their biological effects on wildlife, including pelagic species which may be exposed. In addition, the adsorption property of SWNT may change the bioavailability of contaminants present in various ecosystems. The aim of our study is to identify the effects of the presence of raw SWNT in solution on the toxicity of cadmium (Cd), a toxic metal. To study this SWNT-Cd interaction, we carried out lethality tests using *Daphnia magna* exposed to Cd, raw SWNT and mixture of Cd-raw SWNT. Results of these experiments and a preliminary study of cadmium adsorption on raw SWNT are presented.

Fate and transport of cerium nanoparticles in stream mesocosms differ between pulse and press exposures (PL)

Leanne Baker[1], Ryan King[1], Gregory Lowry[2], Jason Unrine[3] and Cole Matson[1]

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Risk assessment models suggest that the aquatic systems having the greatest potential for exposure to manufactured nanoparticles are streams exposed to wastewater effluent. We determined the fate, transport and biological uptake of cerium oxide nanoparticles in an outdoor stream mesocosm experiment under two different exposure scenarios: a single, large pulse of nanoparticles similar to an accidental spill, and a continuous, low-level addition of nanoparticles, similar to exposure through wastewater effluent. The fate and transport capabilities of nanoparticles under these exposure scenarios was reasonably well described based on current understanding of nanoparticle homoaggregation and heteroaggregation processes, as well as more traditional theories of stream hydrodynamics and colloid science. The pulse addition of nanoparticles led to rapid homoaggregation and deposition, limiting aqueous concentrations and subsequent downstream movement. The continuous press addition of nanoparticles led to higher aqueous concentrations and longer-range transport, likely as a result of the stabilization of particles through heteroaggregation with suspended organic matter. These findings suggest that streams receiving wastewater effluent containing manufactured nanoparticles may experience relatively greater aqueous exposure impacts on aquatic organisms over a greater spatial extent than a similar amount of nanoparticles from an accidental spill.

Silver nanoparticles chemical stress triggers different immune response capacities in early life of sea urchins (PL)

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Like many benthic marine invertebrates, most sea urchins have complex life cycles and develop by means of free-living and dispersive larval stages. This planktonic life lasts

from days to months until metamorphosis and their new benthic existence. While in the plankton, eggs and larvae may face starvation, environmental stresses and widespread pollution sources. Nowadays, nanosilver is used increasingly in consumer products for its antimicrobial properties, and major ecological concerns have arisen over its release into natural waters. Even though many studies have discussed nanosilver toxicity, there has been very little research dealing with comparative effects of these chemicals on various stages of the development of marine invertebrates during and after the chemical stress. We intended to investigate the effects of both dissolved and particulate silver on developmental stages and their physiological responses as a function of time. Thus, our experimental approach included short- and long-term exposures of *Strongylocentrotus droebachiensis* larvae and embryos to four nominal concentrations (500, 100, 50 and 20 $\mu g \cdot L^{-1}$). Experiments were carried out with respect to the timing of major morphogenetic events during the gastrulation and postembryonic development, with some tests carried through to metamorphosis. Microscopic observations in 3-D structure were made *in vivo* by confocal microscopy. The results of this study point towards a strong impairment in coelomocytes and red spherule cell migrations in embryos exposed to sublethal concentrations. In contrast, most larvae exhibited an intense inflammatory response to repair wounded tissues undergoing necrosis at 96 hours of exposure. By that time, oxidative stress was measured (CellRox) and indicated reactive oxygen species (ROS) production mainly under 100 $\mu g \cdot L^{-1}$ and 50 $\mu g \cdot L^{-1}$ of contaminants and starvation. Toxicity of dissolved silver was always more severe than that of silver nanoparticles in acute tests. Also, under silver subchronic stress, larvae undergoing complete metamorphosis showed high mortality in both exposure and recovery times. However, over the experimental period those contaminated with silver nanoparticles mostly struggled passing through metamorphosis. Under nanosilver stress more juveniles were formed in uncontaminated water (recovery time). These findings provide a more precise understanding of nanosilver toxicity and how this chemical newly released into the environment may impact marine invertebrate early life over the time.

Biomarkers in yellow perch exposed to low $\mu g \cdot L^{-1}$ concentrations of nanosilver (PL)

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Nanomaterials are becoming increasingly popular in consumer products due to their unique physico-chemical properties. Nanosilver is found in a wide range of food packaging materials, textiles, electronics, household appliances, cosmetics, and medical devices for its antibacterial properties. As a consequence of increased use, there are concerns that nanosilver is entering waterways from municipal sewage systems in low ($\mu g \cdot L^{-1}$) concentrations. The risks of low concentrations of nanosilver to aquatic ecosystems and the actual mechanisms of toxicity in fish are largely unknown. The toxicity of nanosilver may be due to the nanoparticle itself, the release of silver ions, or a

combination of both. The goal of this research was to determine if nanosilver induces a suite of biomarkers in fish at environmentally relevant (low $\mu g \cdot L^{-1}$) concentrations. Juvenile yellow perch (*Perca flavescens*) were exposed to waterborne dilutions of either a nanosilver suspension or silver nitrate in both acute (96-hour) and chronic (10-day) exposure regimes. Gill, liver, and muscle tissue were collected for measurements of biomarkers and oxidative stress responses including glutathione, lipid peroxidation, metallothionein, and cytochromes P450 1A1 and 3A4/5 (biochemical responses and mRNA expression). Overall, the results of this research help elucidate whether short- and long-term exposures to environmentally relevant concentrations of nanosilver cause sublethal toxicity in fish.

The effects of nanoparticle exposures on the phagocytosis of immune cells (PL)

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[1]University of Alberta

The biomedical community is exploring the use of nanoparticles (NPs) in medical devices for various therapeutic treatments. However, before NPs can be used in *in vivo* applications, their biocompatibility with non-target tissues and cells needs to be rigorously assessed to ensure that only the intended tissues are interacting with the NPs, and at anticipated levels. The toxicological impact on the vertebrate immune system has not been examined to a great extent, despite its importance to overall heath, and current data that demonstrates NPs can both suppress and over-activate various immune responses. The innate immune system is particularly responsive to NPs since innate cells are the first lines of defense to protect from invaders and will likely be the first cell types to encounter NPs. A change in the functionality of innate immunity could affect the manner by which an organism responds to pathogens. Phagocytosis is the major innate immune mechanism to remove pathogens and is conserved across a wide array of vertebrate and non-vertebrate species. Despite its importance for host defense, to date only a few reports have assessed the impact of NPs on cellular phagocytic function. Thus, the aim of this study was to determine the effects of metal-oxide NPs on the phagocytic capacity of the mast cell line, RBL-2H3. Mast cells are resident in most tissues but are particularly located in mucosal linings of the respiratory and gastrointestinal systems, and are principally involved in augmenting the inflammatory response. *Escherichia coli* expressing Green Fluorescent Protein (GFP) was used to stimulate phagocytosis in RBL-2H3 cells, which was measured by flow cytometry. For the first experiment, GFP *E. coli* were opsonized with solutions pre-exposed to NPs at various concentrations (1-200 $\mu g \cdot mL^{-1}$) to determine whether the protein binding capacities of NPs would affect GFP-*E.coli* opsonization and subsequent phagocytosis by RBL-2H3 cells. The second series of experiments tested phagocytosis following pre-exposure of RBL-2H3 cells to various concentrations of NPs (1-200 $\mu g \cdot mL^{-1}$) for various exposure periods (1-24 hours). Using confocal microscopy, phagocytosis was also measured following co-exposure of GFP *E. coli* and NPs to RBL-2H3 cells, to determine whether GFP *E. coli* augmented the

internalization of NPs during phagocytosis and to assess whether internalized NPs correlated with cell apoptosis.

Oxidative stress and regulation of metabolism in spherical zinc oxide nanoparticle-exposed white sucker (*Catostomus commersonii*) (PL)

Neal Callaghan[1]

[1]Mount Allison University

Nanoparticles (NPs) form a trillion-dollar industry, with diverse applications including electronics, cosmetics, engineering and medicine. NPs have previously been associated with oxidative stress and accompanying metabolic regulation in exposed organisms. In this study, white sucker fish were exposed to spherical zinc oxide NPs and probed for hepatic markers of oxidative stress. Metabolic changes were investigated by examining white muscle lactate dehydrogenase (LDH) for differential regulation, including post translational modification, isozyme exchange and allosteric regulation. Results indicated signs of liver oxidative stress with accompanying allosteric inhibition of anaerobic white muscle LDH activity, suggesting that hypoxia was not a result of the oxidative stress, and that the subjects may have been favouring an antioxidant response. These results link two responses to NP exposure, lending insight into systemic interactions leading to an understanding of the organism-level response to the toxin.

Nano vs. neuro: Disruption of gill chemoreceptor function by engineered nanomaterials and associated effects on stress physiology (PL)

Tyson MacCormack[1]

[1]Mount Allison University

We have previously shown that commercially relevant zinc oxide nanomaterials (nZnO) depress heart rate and cause oxidative stress in adult white sucker, *Catostomus commersonii*. We hypothesized that cardiac effects were mediated through gill neuroepithelial cells normally involved in the response to hypoxia and that the observed bradycardia would be accompanied by a proportional decline in cardiac output. To test these hypotheses, we compared cardiovascular function (e.g., cardiac output, stroke volume, etc.) between fish exposed to nZnO and to hypoxia, with or without a prior injection of atropine. Atropine blocks muscarinic acetylcholine receptors and abolishes the hypoxic bradycardia initiated by gill neuroepithelial cells. We also assessed the metabolic impacts of nZnO exposure to determine if the bradycardia was associated with global metabolic depression. nZnO exposure triggered an oxidative stress response in multiple tissues and increased Na/K-ATPase activity and heat shock protein expression in gill. This was accompanied by a decrease in heart rate and no change in resting metabolic rate. Our results suggest that nanoparticles simultaneously initiate a direct biochemical

stress response and a physiological hypoxia response via interactions with gill chemoreceptors. The resulting decrease in cardiac scope and increase in tissue energy demand could substantially reduce aerobic scope, and impact survival in challenging environments. Studies are underway to address these questions and to clarify the risks associated with aquatic nanoparticle exposure.

Fate of silver nanoparticles in freshwater microcosms in realistic conditions (PO)

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The use of silver nanoparticles (AgNPs) in consumer and medical products is still growing while their fate in aquatic environments from their industrial and domestic sources remains undetermined. Moreover, few studies have been conducted in real and complex environmental matrices compared to artificial matrices. A large number of factors indeed could interfere simultaneously in AgNPs behaviour, including O_2 concentration, ionic strength, Cl- concentration, pH, natural organic matter (NOM), cysteine, etc. This study aims to: (a) assess AgNP partitioning (water, NOM, sediment, organisms) in freshwater systems under realistic conditions: natural water and sediment, environmental concentrations (0.1-10 $\mu g \cdot L^{-1}$); and (b) explore the links between silver partitioning and silver bioavailability towards aquatic organisms. Experiments are conducted in simple (standard toxicity assay) and complex conditions (2-L laboratory microcosms) with algae, duckweeds, daphnids, amphipods and chironomids, with natural freshwater and sediment. Different sizes and types of AgNPs are studied: 10 nm and 45 nm, coated by citrate and polyvinylpyrrolidone (PVP). The poster will present total Ag partitioning in microcosms and effects of AgNPs on organisms, while they were exposed in complex media.

Predictive Ecotoxicology: Development and Validation of New Tools to Characterize and Prioritize Chemical Hazards across Fish and Wildlife

Development and application of *in vitro* cell-free neurochemical screening assays to predict adverse outcomes in fish, birds and mammals (PL)

Nil Basu[1], Jessica Head[1], Krittika Mittal[1], Peter Dornbos[2], Adeline Arini[1], Brandon Armstrong[2] and Cheryl Murphy[2]

[1]McGill University, [2]Michigan State University

An estimated 30,000+ current commercial chemicals contain neurotoxic properties. As current animal studies are expensive, time-consuming, and overlook many at-risk organisms, development of a new, high-throughput screening method is required. *In vitro*, cell-free assays have potential as relatively inexpensive, reliable screening mechanisms to flag neurotoxic compounds. Thus, compounds can be prioritized according to their toxicity for more in-depth animal dose-response studies. Given the current lack of screening methods and a limited ability to conduct whole-animal bioassays on at-risk organisms, development of such bioassays is essential for future ecological risk assessment. Here, we report on results from a series of *in vitro* screening assays assessing neurotransmitter receptors (n=10) and enzymes (n=3) associated with essential behaviour and reproduction, including components of the glutamatergic, GABAergic, dopaminergic, serotonergic, cholinergic, and other neurochemical pathways. Such neurochemical receptors and enzymes were isolated from at-risk organisms (n=20) of multiple taxa, including fish (king mackerel, yellowfin tuna, goldfish, rainbow trout, and perch), birds (bald eagle, Japanese quail, chicken, and zebra finch), mammals (river otter, mink, pilot whale, common dolphin, narwhal, ringed seal, and polar bear), and biomedical species (rat, mouse, and human). The isolated neurochemical receptors and enzymes were dosed *in vitro* with a diverse set of 100 potentially neurotoxic chemicals, such as metals, rare-earth elements, pesticides, personal care products, flame-retardants, water/sediment extracts, and others. This was accomplished via development of neurochemical receptor binding and enzyme activity assays in a 96-well plate format. Specifically for receptors, total uninhibited binding of a specific radioligand was compared with radioligand binding in the presence of 50 µM of each potential toxicant. For enzymes, total product was compared with product formed in the presence of 50 µM of each potential neurotoxicant. In this presentation, we will: (a) elaborate on the execution of such cell-free assays and discuss “pros and cons” of our current screening method; (b) explain initial results from our current assays and discuss potential conclusions that can

be drawn from such data; and (c) discuss a strategy by which large volumes of *in vitro* data outputs may be modeled to predict individual-based adverse outcomes pathways.

Application of thermodynamic activity and fugacity to assess the environmental risks of phthalate ester mixtures (PL)

Laura Tupper-Ring[1], S. Victoria Otton[1] and Frank Gobas[1]

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Phthalate esters (phthalates) are a family of chemical substances with varying chemical and physical properties which are used as plasticizers and additives in many consumer products. Because of their widespread use, they have been detected in a variety of environmental media and wildlife species from many locations in the world. While risk assessments for individual phthalates such as DEHP have been conducted previously, the assessment of the cumulative risks of phthalate ester mixtures has remained a challenge. The objective of this research is to perform a cumulative risk assessment of phthalates by using an activity and fugacity-based approach. This approach aims to express exposure and toxicity data in terms of thermodynamic quantities (i.e., the fugacity and activity). An activity- and fugacity-based risk assessment approach makes it possible to include exposure and toxicologically significant concentrations in multiple environmental media in a single risk analysis, hence providing a greater weight of evidence in the risk analysis. Also, by expressing exposure and toxicity data for different phthalates into a common thermodynamic quantity the contribution to the combined toxicity of individual phthalates can be investigated. The research compiled concentrations (n=3721) of 20 phthalate esters in seven environmental and five biological media from a variety of sources (K. Clark, 2010). The study also compiled toxicity data for phthalates. The exposure data revealed that activities of individual phthalates in environmental media range widely from 10^{-15} to $10^{1.8}$, and from 10^{-9} to $10^{-2.5}$ for biological tissues. Exposure activities of phthalates were compared to activities associated with toxic effects to conduct risk assessments for both individual and phthalate esters mixtures. The results are discussed in terms of the ability of thermodynamic quantities to conduct cumulative risk assessments for phthalate esters mixtures.

Bioaccessibility of methylmercury from commonly consumed fish and seafood (PL)

Maia Siedlikowski[1], Mark Bradley[1] and Nil Basu[1]

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Mercury is a global contaminant of concern and concentrations are increasing in many regions. Methylmercury's ability to bioaccumulate and biomagnify through the food web renders it particularly harmful. Fish and seafood consumption represents the

greatest source of methylmercury (MeHg) exposure to people and fish-eating organisms. Here we aim to increase understanding of the bioaccessibility of methylmercury from commonly consumed fish and seafood in North America, including several species native to the continent. To achieve this, bioaccessibilty and bioavailability of MeHg are being determined using an established *in vitro* digestion method. To date, steps have been taken to assess the bioaccessibility of MeHg from 10 different fish species (n=3 individuals per species) with an *in vitro* digestion method that has been modified from the physiologically-based extraction test (PBET). Next we will assess MeHg bioavailability using another existing *in vitro* system. The resulting data from this work will be coupled to an existing epidemiological study we run to better improve our ability to link dietary exposure assessment and MeHg biomarkers, and ultimately assess risks associated with methylmercury contamination of our waterways.

The fathead minnow HPG-L axis as a predictive tool for ecotoxicology (PL)

Theresa Johnston[1] **and Donald Cropek**[2]

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Endocrine disrupting compounds (EDCs) are prevalent in the environment and affect both animals and humans. They often impact the reproductive system by interfering with a portion of the hypothalamic-pituitary-gonadal (HPG) axis. Human development of chemicals for agricultural, industrial, and personal use has led to over 80,000 chemicals with unknown endocrine action. Numerous *in vivo* and *in vitro* testing methods have been developed to screen these chemicals. However, extrapolation of *in vitro* studies to *in vivo* action is not always accurate. The aim of this research was to develop a co-culture of the fathead minnow (*Pimphales promelas*) HPG axis and liver (L) to serve as an *in vitro* system that mimics the interaction of the fathead minnow HPG-axis *in vivo*. This model was expected to show interaction among tissues that resembled a live fish and respond to a known EDC, 17-β trenbolone, more similarly to a live fish than an individual gonad culture. Brain (hypothalamus), pituitary, gonad, and liver tissue were removed from euthanized adult male and female fathead minnow and sliced. Tissues were tested in their ability to communicate with each other in co-culture and separate cultures. After it was shown that the tissues were able to interact *in vitro*, the HPG-L tissues were co-cultured each for male and female FHM. Co-cultures were exposed to 17β-trenbolone at concentrations found in tissues after exposure in an *in vivo* study. Additionally, individual testis and ovary tissues were exposed to the same concentrations. Estradiol, 11-ketotestosterone, and vitellogenin (Vtg) were measured. It was hypothesized that the co-culture would respond to the EDC more similarly to the *in vivo* study results than the individually cultured gonads. Both male and female HPG-L cultures responded to TRB with changes in hormone concentrations that resembled those of a previously reported *in vivo* study (Ankley et al. 2003). After 3 days in culture, male HPG-L tissues responded to the 3 doses of TRB with changes in estradiol (E2), testosterone (T), and Vtg that followed the same trend in hormone changes as the *in vivo* research. In

contrast, the testis cultures only responded to the TRB doses with changes in E2 that had the same trend as the *in vivo* study. These data suggest that a co-culture system with all components of the HPG-L axis represent *in vivo* responses better than individual gonads.

Assessment of the sensitivity of North American fish species to endocrine disrupting chemicals *in vitro* (PL)

Shawn Beitel[1], Jon Doering[1], Sarah Patterson[1], Bryanna Eisner[1] and Markus Hecker[1]

[1]University of Saskatchewan

There is concern regarding exposure of aquatic organisms to chemicals that interfere with the endocrine system. Disruption of the endocrine system can lead to the disruption of sexual development, delayed sexual development, altered hormone levels, intersex, and ultimately reproductive failure. There is a large gap in knowledge about, and a high degree of uncertainty, regarding the sensitivity of wild fish species to endocrine disrupting chemicals (EDCs). However, there are major concerns regarding conducting *in vivo* toxicity testing with wild species, including the large number of animals required, cost and time investment, and ethical concerns (e.g., when working with endangered species). Therefore, the aim of this study was to develop *in vitro* tissue explant assays enabling the assessment of EDCs in wild fish species native to North America. Specifically, gonad and liver explants were used to assess effects of selected EDCs on sex-steroid production and estrogen-receptor mediated processes (vitellogenin (VTG) induction), respectively.

Northern pike (*Esox lucius*), walleye (*Sander vitreus*), and white sucker (*Catostomus commeroni*) were sampled from Lake Diefenbaker, SK, CA, and white sturgeon (*Acipenser transmontanus*) were selected from an in-house stock reared from eggs. Liver tissue was excised and exposed for 24 hours to 17α- ethinylestradiol (EE2). Gonad tissue was excised and exposed for 24 hours to a model inducer (forskolin) and inhibitor (prochloraz) of steroidogenesis. Transcript abundance of VTG, estrogen receptor (ER) α and β in liver tissue were quantified using qPCR. 11-ketotestosterone (11-KT) and estradiol (E2) were quantified in media by use of ELISA. Exposure to EE2 resulted in a concentration dependent increase in VTG in all species, and an increase in ERα in northern pike. Walleye males showed the greatest sensitivity to EE2. Gonad tissues exposed to forskolin showed a concentration dependent increase in 11-KT and E2. Exposure to prochloraz resulted in a decrease of 11-KTand E2. Male and female white sucker showed greatest sensitivity to forskolin, while male and female walleye showed greatest sensitivity to prochloraz. The seasonal time point during which gonad explants were excised and exposed had an impact on the potency and magnitude of responses, resulting in a seasonal effect on sensitivity. Also, gonad explants from these species were found to have greater sensitivity than responses previously reported for *in vitro* explants of other fish species such as the fathead minnow (*Pimephales promelas*), and stable cell lines currently used as screening applications to detect chemicals that might disrupt the

endocrine system. Therefore, current approaches that use stable cell lines or tissue explants from standardized small bodied laboratory species might not be protective of some wild fish species. Future research is required to investigate whether these *in vitro* explant assays are predictive of *in vivo* effects in wild fish species.

Functionality of aryl hydrocarbon receptors (AhR1 and AhR2) of white sturgeon (*Acipenser transmontanus*) and implications for the risk assessment of dioxin-like compounds (PO)

Jon Doering[1], Reza Farmahin[2], Steve Wiseman[1], Sean Kennedy[2], John Giesy[1] and Markus Hecker[1]

[1]University of Saskatchewan, [2]Environment Canada

Worldwide, populations of sturgeons are endangered, and it is hypothesized that anthropogenic chemicals, including dioxin-like compounds (DLCs), might be contributing to the observed declines in populations. DLCs elicit their toxic action through activation of the aryl hydrocarbon receptor (AhR), which is believed to regulate most, if not all, adverse effects associated with exposure to these chemicals. Currently, risk assessment of DLCs in fish uses toxic equivalency factors (TEFs) developed for the World Health Organization (WHO) that are based on studies of embryo-lethality with salmonids. However, there is a lack of knowledge regarding the sensitivity of sturgeons to DLCs, and it is uncertain whether TEFs developed by the WHO are protective of these fishes. Sturgeons are evolutionarily distinct from salmonids and the AhRs of sturgeon differ from those of salmonids. Therefore, this study investigated the sensitivity of white sturgeon (*Acipenser transmontanus*) to DLCs *in vitro* by use of luciferase reporter gene assays using COS-7 cells transfected with AhR1 or AhR2 of white sturgeon. Specifically, activation and relative potencies (RePs) of 2,3,7,8-TCDD, 2,3,7,8-PeCDF, 2,3,7,8-TCDF, PCB 126, PCB 77, and PCB 105 were determined for each AhR.

It was demonstrated that white sturgeon express AhR1s and AhR2s that are both activated by DLCs with EC_{50}s for 2,3,7,8-TCDD lesser than any other AhR of vertebrates tested to date. Both AhRs of white sturgeon had RePs for polychlorinated dibenzofurans (PCDFs) more similar to TEFs for birds, while RePs for polychlorinated biphenyls (PCBs) were most similar to TEFs for fishes. When RePs developed for white sturgeon in this study were compared against TEFs for fishes used by the WHO (by use of measured concentrations of select DLCs in tissues from white sturgeon in British Columbia, Canada), toxic equivalencies (TEQs) calculated by use of TEFs were approximately 10-fold lesser than TCDD Equivalents (TCDD-EQs) calculated by use of RePs developed for white sturgeon. TCDD-EQs calculated for endangered populations of white sturgeon were within ranges known to cause adverse effects in other fishes, including other species of sturgeons. Therefore, TEFs used by the WHO might not adequately protect the white sturgeon, illuminating the need for additional investigation into the sensitivity of these fish to DLCs.

Design and validation of degenerate primers for PCB-interacting genes conserved in animals (PO)

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In the context of ecotoxicology, designing degenerate primers for xenobiotic-interacting genes conserved across phylogenetically distant taxa is a novel approach to assessing the targeted-transcriptome response of a diverse range of animals to pollutants. Among other reasons, degenerate primers are used to amplify genes in non-model organisms—animals without known sequence information—and/or to amplify a target gene(s) in more than one species. A set of 71 PCB-interacting genes conserved in animals (≥49.5% nucleotide conservation per region selected) was determined and used as the gene set for degenerate primer development. Consensus-Degenerate Hybrid Oligonucleotide Primer (CODEHOP) is a degenerate primer design strategy based on all the possible codons for the conserved regions of protein multiple sequence alignments. Using CODEHOP's default parameters, one primer set (forward and reverse) for each PCB-interacting gene was chosen based on amplicon length, low degeneracy, and melting temperature. With these degenerate primers, a set of conserved PCB-interacting genes was amplified in *Hexagenia limbata*, a non-model, standard toxicological organism and common bioindicator species, and in *Danio rerio*, a model, standard toxicological organism. Amplicons of these genes were analyzed, sequenced, and verified by blasting sequences against the local database of sequences used in this study. The success of amplifying this set of conserved PCB-interacting genes in divergent organisms creates a toolkit to probe the targeted transcriptome of a wide range of species, from vertebrates to invertebrates, from model organisms to non-model organisms, from bioindicator species to conventional toxicological organisms, both naturally-exposed and laboratory-exposed to PCBs. Ultimately, this tool kit will provide "an early warning system" for any taxa exposed to PCBs and will help ecotoxicologists identify sites of biological concern in a timely and cost-effective manner.

Development of a tissue co-culture model of the chicken Hypothalamus-Pituitary-Gonadal-Liver (HPG-L) axis for *in vitro* toxicological evaluation (PO)

Krittika Mittal[1], Theresa Johnston[1] and Nil Basu[1]

[1]McGill University

Exposure to Endocrine Disrupting Chemicals (EDCs) such as pesticides and industrial chemicals can cause changes in reproductive physiology, neuroendocrinology and metabolism. The HPG-L axis is an essential reproductive pathway responsible for development and function of the reproductive organs and controlling behaviours of reproduction. Disruptions in this pathway can cause severe endocrine imbalance and sex-ratio disturbances, which could have population-level impacts. Field- and whole-

organismal studies have shown that EDCs adversely affect avian species such as chickens, quails and bald eagles. While offering compelling evidence of harm, organismal studies involve sacrificing a large number of animals and are time consuming and expensive. In recent years there has been great interest in developing *in vitro* systems to study biologically relevant disturbances in interconnected pathways composed of complex biochemical interactions and small molecules that control communication between cells. Among the various *in vitro* approaches being explored, tissue explants show great promise. For example, explants from neonatal rats and embryonic chicken retina had superior histology, showed remarkable retention of architecture and were more similar to freshly isolated tissue than 2-D cell culture. Given the promise of tissue explants as an alternate *in vitro* model, the objective of this study is to establish and sustain an explant co-culture of the chicken HPG-L axis. Tissues from white leghorn chicken embryos day 19 were sectioned and cultured at pH 7.4, 37°C and 5% CO_2 in DMEM, IMDM and Medium 199. Media was changed daily, every other day and weekly. Tissue viability was assessed by MTT assay on day 1 and at the end of the culture period (day 12). Cultures with greater than 80% viability on day 12 were considered successful. Preliminary data suggests that DMEM with every other day media change was best suited for hypothalamus and liver, whereas DMEM with weekly media change was best suited for gonads. Studies to assess functionality of the tissues and establish the co-culture are ongoing. Following the establishment of suitable culture conditions, tissues will be dosed with a range of EDCs. Understanding the effects of contaminants on the HPG-L axis is important in determining the detrimental effects that EDCs have on humans and wildlife at organismal levels, and this *in vitro* model could serve as a valuable screening tool to study these interactions.

Risk Assessment and the Nuclear Fuel Cycle

Environmental regulation of nuclear facilities in Canada (PL)

Malcolm McKee[1] **and Barbara Dowsley**[1]

[1]Canadian Nuclear Safety Commission

The Canadian Nuclear Safety Commission (CNSC) regulates the use of nuclear energy and materials to protect the health, safety and security of Canadians and the environment. All nuclear-related facilities and activities (e.g., uranium mines and mills, nuclear power plants, waste management facilities) require a licence from the CNSC. Environmental risk assessment (ERA) is applied at the environmental assessment stage as a predictive tool for optimizing facility design and determining (a) whether the facility is likely to result in a significant adverse effect as required by Canadian Environmental Assessment Agency and/or (b) whether the proponent will make adequate provision for the protection of the environment and the health and safety of humans, as required by the *Nuclear Safety and Control Act*. Should the facility proceed to licensing, ERA predictions form part of the basis upon which the facility is to operate and against which the facility performance is assessed. Furthermore, the ERA serves as the primary tool for identifying and developing the key elements of the emissions and receiving environment monitoring programs, and identifying the key "environmental aspects" within the facilities environmental management system (e.g., ISO 14001). Compliance and assessment activities are enhanced by the requirement to assess performance against previous predictions and update the ERA at minimum on a 5-year cycle. The ERA update incorporates the accumulated facility and environmental data and new science. The site-specific ERA model becomes an increasingly robust adaptive management tool as the assessment and predictive power is improved with the refinement of key model assumptions, the accumulation of site-specific data, the completion of specialized studies and the incorporation of new science. The results of the updated ERA are incorporated into the compliance program as appropriate to ensure continuous protection of the environment.

Environmental pathways modelling for Class 1 nuclear facilities and uranium mines and mills in Canada: A discussion of the interactions between environmental protection programs and environmental risk assessment (PL)

Lynnae Dudley[1], Janeen Tang[1] and Ronald Nicholson[1]

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In Canada, environmental pathways modelling is integral to the licensing process for Class 1 nuclear facilities and uranium mines and mills regulated under the *Nuclear Safety and Control Act* (NSCA) administered by the Canadian Nuclear Safety Commission (CNSC). Environmental pathways models are used in environmental risk assessments to evaluate the potential effects of proposed activities on humans and non-human biota from exposure to radiological and non-radiological constituents in environmental media. Environmental assessments have shown that understanding aquatic pathways is often crucial when assessing the environmental fate of constituents associated with these nuclear-related activities in Canada. Potential releases to the aquatic environment include treated liquid effluents from Class 1 facilities or ore processing, treated mine water and dewatering discharges, and treated leachate from ore stockpiles, mine rock, and tailings management facilities. Environmental pathways models provide quantitative estimates of concentrations of, and exposures to, constituents of potential concern that are compared to relevant benchmarks protective of ecological and human health, to assess potential risk.

The development of environmental pathways models is an iterative process. Initially, environmental pathways models may rely on conservative assumptions to address uncertainties and ensure that potential risks are not underestimated. The models are refined over time as key environmental processes and parameters are identified and investigated to provide site-specific values. The development of site-specific model parameters and validation of the model at multiple locations reduces uncertainties and improves confidence in model predictions. Within the CNSC's Environmental Protection Framework, evergreen risk assessments are informed by monitoring programs, support the regulator's decisions, and are a key element of environmental assessments conducted under both the NSCA and the *Canadian Environmental Assessment Act, 2012*. For licensees, environmental pathways modelling can provide additional benefits including playing pivotal roles in project development prior to licensing and in designing effective Environmental Protection Program components for a licensed activity. Examples will be presented to illustrate how the Environmental Protection Program can be used to support the development of the environmental pathways model and inversely, how the model can be used as a planning and environmental management tool to inform decision making early in a project's development.

Approach to the assessment of radiological dose to non-human biota (PL)

Stacey Fernandes[1], Katherine Woolhouse[1] and Douglas Chambers[1]

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To assess the potential impact of exposure to radionuclides on ecological receptors, the dose to the receptor must be estimated. This dose can then be compared to a benchmark value to determine the potential for an effect. In this presentation, the approach to conducting a radiological dose assessment for ecological receptors (also called non-human biota) will be outlined. A dose assessment provides an estimate of the amount of radiation energy that might be absorbed as a result of exposure. For radioactivity, both the external dose and internal dose (arising from radioactivity taken into the organism through ingestion or inhalation) must be considered. In 2012, the Canadian Standards Association (CSA) released a document outlining the approach for the assessment of environmental risk at nuclear facilities (N288.6). The selection of dose coefficients and appropriate weighting factors, as recommended by N288.6, will be discussed. Examples will be provided that focus on biota potentially exposed to the uranium-series radionuclides in the aquatic environment. The implication of different calculation approaches, such as biota concentration ratios used in the ERICA Tool and the use of intake rates and transfer factors, will be evaluated. The current status of dose assessment within the international community will also be examined.

Kiggavik Project environmental risk assessment (PL)

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[1]AREVA Resources Canada Inc.

AREVA Resources Canada Inc. (AREVA) is proposing to construct, operate and decommission a uranium mine and mill, called the Kiggavik Project (Project), in the Kivalliq Region of Nunavut. The Project is located approximately 80 km west of the community of Baker Lake. The Project is currently in the Environmental Assessment process under the Nunavut Impact Review Board (NIRB), with the Canadian Nuclear Safety Commission (CNSC), Transport Canada and other government agencies providing technical review and subsequent authorizations. Environmental assessment (EA) is used to identify possible Project-environment interactions and their potential to result in an environmental effect(s). Mitigation to reduce potential effects is then considered and the significance of the remaining residual effects determined. AREVA believes this process should be iterative so that a good understanding of the local environment and site-specific constraints can influence design and mitigation and the subsequent evaluation of potential environmental effects. This iterative process leads to a selection or narrowing of Project alternatives and further refinement of Project design. An overview of the Project, including environmental and human health risk assessments with a focus on the aquatic

environment, integration of Inuit Qaujimajatuqangit (IQ), and predicted residual effects and assessment outcomes will be presented.

The influence of sediment characteristics on uranium (VI) bioavailability to the freshwater midge, *Chironomus dilutus* (PL)

Sarah Crawford[1] **and Karsten Liber**[1]

[1]University of Saskatchewan

The anticipated increase in demand for nuclear power production would result in the increased mining of uranium (U), which would raise the potential for increased U contamination of adjacent aquatic ecosystems. The interactions between dissolved U and solid phases can result in concentrations of U in sediment that are several orders of magnitude higher than those in the water column. The tendency of U to accumulate in sediments makes benthic invertebrates among the most highly exposed organisms. Benthic invertebrates are also an important group of organisms used for the evaluation of metal impacts in aquatic environments, and for the development of sediment quality guidelines in Canada and equivalent criteria around the world. The goal of this research is to quantify the influence of key sediment characteristics on U bioavailability, and hence on toxicity of sediment-associated U to a model freshwater benthic invertebrate.

Larvae of the freshwater midge, *Chironomus dilutus*, were exposed for 10 days to both field-collected and formulated sediments spiked with U (aged for 20 days) to determine differences in U bioaccumulation from sediment with different physicochemical properties. The physicochemical characteristics of sediment that were examined in this research were particle size distribution (especially clay content) and organic matter (OM) content, as they are predicted to alter the partitioning and bioavailability of U and other metals associated with sediment. The binding capacity of the field and formulated sediments were quantified in laboratory sorption tests to aid in the interpretation of bioaccumulation data from separate spiked sediment tests. Results from these studies have demonstrated quantitatively that with similar concentrations of U in sediment, the actual amount of U available for biological uptake is influenced by the composition and binding capacity of the sediment. Formulated and field sediments with higher clay or OM content significantly reduced concentrations of U in the dissolved phases (pore water and overlying water), with a corresponding decrease in bioaccumulation of U in exposed *C. dilutus* larvae (R^2=0.76, $p<0.05$). Thus, the sorptive characteristics of key physicochemical properties of sediment may possibly be used to generate a simple predictive model that could be used in the risk assessment of U contaminated sites and possibly in the development of site-specific sediment regulatory guidelines or objectives.

Boreal Watershed Management Strategy – Eastern Athabasca Regional Monitoring Program (PL)

Kevin McCullum[1]

[1]Saskatchewan Ministry of Environment

The Ministry of Environment in Saskatchewan embarked on a Cumulative Effects Monitoring Program in 1994 as a result of recommendations from public hearings surrounding Northern Saskatchewan Uranium mining. Data from this program has been collected from 1994 to 2007, where the primary focus included temporal and spatial analysis of water chemistry, sediment chemistry, fish chemistry, and radon. In 2008, the program was suspended pending program funding and program review. In 2011, a new collaborative program was created to include expertise from industry, academia, and government. This new collaborative program was designed to sample Northern Saskatchewan, focusing on baseline conditions in pristine areas and temporal changes in other regions. The objective of this program is to identify the ecological integrity of the northern watersheds. The program is known as the Boreal Watershed Management Strategy. Baseline studies will allow the ministry and key stakeholders such as industry and northern residents to evaluate our current conditions, protect the environment, and facilitate in the development and growth of northern resources. Monitoring programs and partnerships have been formed to work on and address Clean Water Management Strategies, Aquatic Ecosystem Health, Clean Air Management Strategies, Land Management Strategies, Terrestrial Ecosystem Health (Biodiversity Strategies), First Nations and Métis Traditional Knowledge, and Legacy Data Analysis.

One partnership that has been identified as a flagship program is a project under Biodiversity called the Eastern Athabasca Regional Monitoring Program (EARMP). This program is coordinated in conjunction with Cameco, AREVA, and Northern communities including First Nations and Metis Communities (Black Lake Denesuline First Nation, Camsell Portage, Fond du Lac Denesuline First Nation, Stony Rapids, Hatchet Lake Denesuline First Nation/Wollaston Lake, Uranium City) and the Athabasca, Keewatin Yatthe and Mamawetan Churchill River Health Regions. This program includes two sub-programs: a community program and a technical program. The objective of the community program is to monitor traditionally harvested country foods by collecting and testing water, fish, berry, and mammal chemistry from seven communities in northern Saskatchewan. The objective of the technical program is to determine whether impacts are occurring in aquatic environments in northern Saskatchewan by analyzing both the physical and biological components of waterbodies located downstream of converging watersheds that are exposed to mining and milling operations in the Athabasca Region. It is designed as a technical program to study water chemistry, fish chemistry, berry chemistry, and mammal chemistry, and as a community program to study traditional foods. The ERAMP program is structured to be open, transparent, interpretive and flexible in monitoring needs.

Population- and community-level assessments of benthic invertebrates near Chalk River Laboratories (PL)

David Rowan[1], Jamie Carr[1], Renee Silke[1] and Matt Bond[1]

[1]Atomic Energy of Canada Ltd.

The Ottawa River has received effluent from Chalk River Laboratories as a result of nuclear research and development and operations for more than 60 years. Recent interest in the potential impact of these historical releases and the possible need for remediation of a small region immediately downstream from the release point has led to comprehensive studies to assess ecological risk. In this paper, we present the results of an extensive survey of sediment radionuclide concentrations and benthic community and population studies in the Ottawa River in the vicinity of the Chalk River Site. Statistical comparisons of community and population metrics between upstream, contaminated and downstream sites, showed no significant differences. We also found no statistically significant relationship between abundance, biomass or size of *Hexagenia* in relation to radiation dose or to sediment concentrations of cesium-137 (137Cs), the predominant anthropogenic radionuclide. Our results suggest that contamination near the reactor outfall has no significant impact on the Ottawa River benthic community or the *Hexagenia* population.

A method for the quantitation of hydrazine in liquid effluents released from CANDU nuclear power plants (NPPs) (PL)

Hemendra Mulye[1] and Slobodan Jovanovic[1]

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Hydrazine is a water treatment chemical used in steam generators of nuclear power plants (NPPs) for protection against corrosion. Hydrazine functions as a scavenger of oxygen in the steam generator and balance of the plant through a reduction reaction, with the formation of nitrogen as a major transformation product. Other transformation products include water, ammonia, and hydrogen peroxide. Discharges of hydrazine to the environment can occur through partial removal of water with replacement by fresh demineralized water to maintain a constant low level of dissolved solids. This water removal, called “blow down”, becomes part of the final effluent discharged from NPPs and contains hydrazine because it is used in stoichiometric excess to ensure removal of dissolved oxygen from the make-up water used to compensate for the water lost during blow down. In 2011, the federal Ministers of the Environment and of Health published a screening assessment of hydrazine and, as a result, hydrazine was designated as a toxic substance under the *Canadian Environmental Protection Act, 1999* (CEPA), which resulted in its addition to Schedule I of CEPA, and an associated review of the options for controlling risks to human health and/or to the environment. Therefore, a Pollution Prevention Notice (P2 Notice), as a management option under the federal Chemical

Management Plan, was published by Environment Canada which would require power plant operators to sample, measure, report, and monitor levels of hydrazine in effluents released to the environment. A Federal Environmental Quality Guideline for hydrazine was published by Environment Canada in 2013, which specifies numerical values for the protection of freshwater and marine/estuarine life from the adverse effects of hydrazine. The Canadian Nuclear Safety Commission (CNSC) laboratory has developed a novel, sensitive, and accurate method for the sampling and analysis of hydrazine in feed water samples at NPPs. This method for determination of hydrazine employs ion chromatography coupled with an amperometric detector which is 500 times more selective for hydrazine and sensitive enough (limit of quantitation 0.1 $\mu g \cdot L^{-1}$) to monitor compliance with the federal guideline. In addition, the stability of hydrazine in environmental waters was studied to establish proper sample preservation. This paper will present the methodology and results of a hydrazine sampling program at one NPP site in Canada.

Estimating sediment deposition rates in lakes downstream of closed uranium mines (PL)

Cheryl Wiramanaden[1], Cynthia Russel[1], Ian Ludgate[2] and Debbie Berthelot[3]

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The Serpent River Water Monitoring Program (SRWMP) was designed to be a care and maintenance monitoring program for the recovery and improvement of the aquatic environment downstream of several closed uranium mines in Elliot Lake, Ontario. During the establishment of the monitoring program, the frequency of monitoring was determined such that sediment and benthic invertebrate community recovery would be detectable. The rate of recovery was based on the estimated time necessary to deposit 1 cm of fresh sediments. Benthic invertebrate community indices are expected to indicate improvement more slowly than sediment, since benthic macro-invertebrates inhabit sediment to deeper than 1 cm. Sediment deposition was estimated to occur at a rate of 2 mm per year on the basis of sediment dating using pollen and diatomaceous shells to indicate time markers (McKee et al. 1987) at one lake during mining operation. On the basis of a 2 mm per year sedimentation rate, the SRWMP monitoring frequency was established at once every five years. The mines closed in the early 1990s. Since the SRWMP implementation in 1999, water quality and metal loadings to the near-field lakes have significantly improved to below water quality guidelines; however, sediment quality has generally shown a lack of measureable improvement. The 2 mm per year sedimentation rate was determined in 1984, when mines were still operating. Therefore, in the present study, three near-field lakes were selected with expected varying productivity in order to re-evaluate sediment deposition rates. Sediment deposition rates were determined using two approaches: sediment traps to assess current deposition rates at SRWMP benthic monitoring stations, and sediment core profiles, collected from the deepest parts of the lakes, to investigate historical sediment quality. Sediment trap

determined that deposition rates in Lake One ranged 0.40 to 0.44 mm per year. The sediment core profiles of non-migratory analytes (e.g., titanium, caesium-137) were used in combination with historical knowledge of mining activities in a weight-of-evidence approach to establish a timeline with sediment-core depth. The use of time-markers along the core profile allowed the determination of a sedimentation rate estimate of 0.6 mm per year which was in good agreement with the sediment trap deposition rates. Other lakes in the study were estimated to have deposition rates ranging from 0.3 to 0.7 mm per year, indicating that the frequency of monitoring (based on 2 mm per year) is too frequent for measurable improvement. In the present study, sediment deposition is slow and it would take between 13 and 30 years for 1 cm of fresh sediment to deposit on these lakes.

CNSC risk determination and management of thermal discharge on fish and fish habitat resulting from the operation of nuclear power plants (PO)

Changhai Ji[1]

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The operation of nuclear power plants (NPP) with once-through cooling systems necessitates the release of large volumes of heated cooling water, referred to as thermal discharge. This discharge poses a potential risk to the aquatic environment, especially to sensitive fish species and their associated spawning habitat. The thermal discharge is a hazardous substance under *Nuclear Safety and Control Act* (NSCA). The NSCA and associated regulations require that operators make adequate provision for environmental protection and take all reasonable precautions to control releases of hazardous substances and protect the environment. Thermal discharge is a deleterious substance under the *Fisheries Act* (Section 36(3)), which prohibits the deposit of deleterious substances into waters frequented by fish unless authorized by federal regulations. Effects to fish and fish habitat from thermal discharge are evaluated as part of CNSC staff's review of operator's environmental assessment (EA) studies and results of EA follow-up monitoring programs. Thermal discharge has been identified as a concern for potential fish mortality by Canadian Nuclear Safety Commission (CNSC) and Environment Canada staff. Therefore, there is a need for determination of the risk level of thermal discharge on fish and fish habitat. CNSC staff take a consistent approach in determining the risk based on lines of evidence such as EA predictions, on-going environmental monitoring results, site-specific fish studies, fish spawning habitat mapping, and technical support from other federal agencies. The CNSC risk-informed decision-making process is used as guidance for determining the level of risk and the need for site-specific mitigation measures to reduce the risks to an acceptable level. The results of CNSC staff risk determination are used for risk management purposes and could trigger the need for CNSC regulatory actions, such as requesting studies that investigate mitigation measures to reduce effects from thermal discharge. CNSC's risk determination and management approach to thermal discharge is illustrated by a case study.

Amphibian Models for Ecotoxicological Research and Regulatory Applications

Bringing the first true frog genome to ecotoxicology (PL)

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Frogs have an undeniably critical role as sentinel species, food source, and ecosystem services provider. They have been central in the identification of environmental contaminant effects on steroid and thyroid hormone endocrine systems and are exquisite toxicological indicators in aquatic and terrestrial ecosystems. Despite their central importance, a paucity of molecular resources exists particularly for the "true frogs" or Ranidae that represent the greatest number of Amphibia. We have recently begun to sequence the first Ranid genome in its entirety, providing unprecedented tools for tackling comparative ecotoxicology and identifying vulnerable biological systems important in ecotoxicology. The latest results of this endeavor will be presented with examples of ecotoxicological applications.

Characterization of adverse outcome pathways of ethynylestradiol in *Xenopus laevis* (PL)

Markus Hecker[1], Amber Tompsett[2], Eric Higley[1], John Giesy[1] and Steve Wiseman[1]

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Little is known about the molecular key events that drive the development of altered phenotypes in amphibians after exposure to estrogenic chemicals during the period of sexual determination and differentiation. This study investigated the transcriptome-level effects of exposure to 17α-ethynylestradiol during this sensitive period in *Xenopus laevis* by use of Illumina sequencing coupled with RNA-Seq analysis. Molecular toxicity data was correlated with apical outcomes, such as altered gonadal development and metamorphosis, in an attempt to describe adverse outcome pathways for the exposure of amphibians to estrogens. Exposure to EE2 during sexual determination and differentiation affected the transcriptome of genetic male *X. laevis* at Nieuwkoop-Faber stage 53 in a manner that could be linked with phenotypic data. Specifically, pathways and processes that were impacted included steroid and xenobiotic signaling and metabolism, steroid biosynthesis, thyroid hormone signaling and metabolism, and testicular development and spermatogenesis. Some of these pathways, such as thyroid hormone signaling and metabolism and testicular development, could be

linked with observed biological effects on gonadal phenotypes and metamorphosis that were observed in a group of frogs that was exposed to EE2 throughout larval development. In conclusion, analysis of the transcriptome of exposed individuals by use of Illumina sequencing coupled with RNA-Seq was useful for determining the molecular changes that might drive these biologically relevant effects.

Lethal and sublethal effects of acute exposures to phthalates in Western clawed frog larvae (PL)

Valérie Langlois[1], Justine Mathieu-Denoncourt[1], Christopher Martyniuk[2], Jennifer Loughery[3], Viviane Yargeau[4] and Shane de Solla[5]

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Phthalates are plasticizers used in a variety of polymers to increase their flexibility and are now ubiquitous in the environment. As few studies have focussed on the adverse effects of these chemicals in aquatic species, this project aimed at elucidating the effects of phthalates in amphibians. In this study, Western clawed frog (*Silurana tropicalis*) tadpoles were acutely exposed to water spiked with monomethyl (MMP), dimethyl (DMP) and dicyclohexyl (DCHP) phthalates from Nieuwkoop-Faber stages 12 to 46. DMP and DCHP were embryotoxic, and induced larval mortality, malformation, and the up-regulation of a cellular stress-related gene. The diesters DMP and DCHP also hindered tadpole development and altered the expression of dio1. MMP had only a slight effect on development at 1500 $mg \cdot L^{-1}$.

To characterize sublethal effects of phthalates in tadpoles, gene expression analysis was conducted using a custom microarray for *S. tropicalis*. Differentially expressed unique probes in DCHP, DMP, and MMP were as follows: 3575, 3059, and 2544 ($p<0.05$; false discovery rate adjusted). There were 274 unique probes in common among all three treatments. Noteworthy was that individuals treated with DMP and DCHP showed expression patterns that were most different from those of control animals or animals exposed to MMP, and this may reflect the differences observed between the phthalates in terms of tadpole development. Parametric analysis of gene set enrichment revealed that biological processes related to xenobiotic metabolic processes, sulfate assimilation, organic acid metabolic process, and response to toxins were affected with all three phthalate treatments. This is the first study to investigate the effects of these phthalates in amphibians. These assays provided valuable data that will help better assess and manage the risks brought on by the production and the release of phthalates into the environment.

Arsenate exposure-responsive gene expression profile in Western clawed frog, *Silurana tropicalis* (PL)

Jing Zhang[1], Laura Gibson[1], Jennifer Loughery[2], Christopher Martyniuk[3], Mark Button[1], Guilhem Caumette[1], Iris Koch[1], Kenneth Reimer[1], William Cullen[4] and Valérie Langlois[1]

[1]Royal Military College of Canada, [2]University of New Brunswick / Canadian Rivers Institute, [3]University of Florida, [4]University of British Columbia

Arsenic is an abundant element in the Earth's crust and a potent environmental pollutant. Uncontrolled aquatic arsenic content is toxic to plants and animals. In contrast to the extensive studies on arsenic-dependent human health issues, knowledge of the impact of arsenic exposure on aquatic amphibians is fairly limited. The current study therefore addresses this knowledge gap by examining arsenic-responsive gene expression profile in the Western clawed frog (*Silurana tropicalis*). Arsenic toxicity is dependent on its speciation. In general, inorganic arsenic species is more toxic than the organic forms, and arsenite (AsIII) exhibited higher toxicity than arsenate (AsV) in organisms. Arsenate is structurally similar to phosphate. Thus, extracellular arsenate can be transported through phosphate carriers in cellular membrane into cell and compete with phosphate, thereby interfering with normal cellular functions. Arsenite, on the other hand, interacts with proteins through thiol binding. Arsenite is also capable of introducing oxidative species production. Thus it is reasoned that arsenic compounds are able to induce a response in the transcriptome through the aforementioned interactions with cellular protein components. Arsenic detoxification includes methylation-dependent biotransformation. Arsenic methylation shares the same methyl source with DNA methylation, potentially affecting chromatin structures.

Frogs were exposed from Nieuwkoop-Faber (NF) stage 12 to 66 to three inorganic arsenate treatments: 0, 0.5 (low) and 1 (high) $\mu g \cdot g^{-1}$. Gene expression profiling was used to explore the arsenic-dependent gene expression pattern in the earliest NF stage where arsenic species could be detected (NF46). Pathway analyses such as gene set enrichment analysis (GSEA) and sub-network enrichment analysis (SNEA) were implemented to investigate the molecular consequence to arsenic exposure in the frogs. Genes related to histone re-modelling, fatty acid and lipid metabolism, bile acid synthesis as well as reproduction were over-represented in both low and high arsenate treated animals. Consistent with what is known about arsenic and oxidative stress, exposure to arsenate differentially altered the expression levels of the genes linked to antioxidant defence pathways. Real time RT-PCR was used to further investigate the impact of arsenate exposure on reproductive hormonal control. Agreeing with the enrichment results from microarray analysis, the androgen receptor (ar) mRNA and other genes that regulate androgen biosynthesis, such as 3-oxo-5α-steroid 4-dehydrogenase 2 (srd5α2) and steroidogenic acute regulatory protein (star), decreased in NF46 tadpoles under both arsenic conditions. Real time RT-PCR data also suggested stage-dependent expression patterns for these genes in response to arsenic. Overall, our study demonstrates that

single arsenic compound exposure can affect a variety of biological processes in Western clawed frogs.

Effects of a glyphosate-based herbicide and nutrient additives on amphibians in a whole ecosystem experiment (PL)

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The use of herbicides in the agricultural sector is common practice nowadays all over the world. Herbicides help to reduce interspecific competition among plants and thereby enhance crop growth, quality and volume to produce more food for the world's growing population. However, herbicide residues can be toxic to the non-target wildlife species in the environment as well as to the consumers of agricultural food products. Glyphosate-based herbicides are the most commonly used herbicides in agricultural areas and are generally considered to be relatively non-toxic. However, several laboratory and mesocosm studies indicate that a variety of glyphosate-based herbicide formulations can be toxic to amphibian larvae at concentrations that approach predicted worst-case exposure scenarios. In contrast, an increasing number of field studies have shown that these herbicides have little impact on larval amphibian survival, development and growth in typical forestry scenarios.

To investigate the ecotoxicological effects of a glyphosate-based herbicide in an agricultural exposure scenario, we conducted a large scale whole ecosystem experiment. Twenty-four small wetlands were split in half with an impermeable plastic barrier so that one side of experimental wetlands was treated and the other served as an untreated control. The glyphosate-based herbicide Roundup WeatherMAX® was applied at two concentrations, one that approximates the highest concentrations observed in agricultural wetlands ("low", or L=210 µg a.e.·L^{-1}) or a concentration similar to that which is predicted under a reasonable worst-case exposure scenario ("high", or H=2,880 µg a.e.·L^{-1}). Herbicide treatments were applied either alone or in combination with nutrient additions in replicate (n=6). The experimental concentrations were confirmed by a recently developed liquid chromatography-tandem mass spectrometry (LC/MS-MS) method using an Acclaim® WAX-1 multimode (reversed-phase and weak anion-exchange combined) separation column. We monitored larval survival, development and growth of wood frog larvae (*Lithobates sylvaticus*) over two years, as well as the abundance of green frog (*L. clamitans*) and spring peeper (*Pseudacris crucifer*) larvae over one year.

The results from the majority of treatments indicated that the application of a glyphosate-based herbicide alone or in combination with nutrient enrichment had very little negative impact on the survival, growth or development of amphibian larvae, except in the high glyphosate and nutrient enrichment where survival was much lower in the *in situ* cages on the treated sides of wetlands. This work provides a more comprehensive

understanding of the effects of herbicide application and how natural wetland ecosystems recover from herbicide application and nutrient enrichment.

Development of an *in vitro* culture system to test the potential endocrine-disrupting effects of naphthenic acids on wood frog (*Lithobates sylvaticus*) tadpole ovarian development (PL)

Maria Vu[1], Laia Navarro-Martín[1], Juan Manuel Gutierrez-Villagomez[1] and Vance Trudeau[1]

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One major contributor to the global decline of amphibians is the accumulation of environmental pollutants to which amphibians are most susceptible due to their moist and permeable skins. Recently, a group of chemicals found in the Alberta oil sands called naphthenic acids are posing threats to wildlife due to widespread leakage from tailing ponds into aquatic habitats. This study aims to determine the potential endocrine-disrupting role of naphthenic acids on sexual development in wood frogs (*Lithobates sylvaticus*). As a step towards this goal, an *in vitro* organ culture system for tadpole ovaries was developed, enabling direct gonad-specific effects to be examined while excluding influences from external physiological systems. A one-week incubation was carried out in which the gonad-kidney complex of tadpoles was exposed to naphthenic acids, ethinylestradiol (EE2) and triiodothyronine (T3). Histological images reveal successful preservation of oocytes using this *in vitro* method where cultured oocytes exhibited no significant differences in diameters ($p=0.618$) and areas ($p=0.359$) when compare to uncultured conditions. Treatments with 0.5 nM of T3 did not produce any significant effects, but ovaries exposed to 10 $\mu g \cdot L^{-1}$ of EE2 were accompanied by significantly higher degrees of oocyte atresia ($p=0.002$) and reductions in oocyte areas ($p=0.008$) and diameters ($p=0.008$), which are suggestive of ovarian regression. No significant alterations were detected in ovaries exposed to 1.5 $mg \cdot L^{-1}$ of naphthenic acids, though a significant reduction in oocyte area was examined at a concentration of 3 $mg \cdot L^{-1}$ ($p=0.036$). These findings have biological implications for understanding how naphthenic acids disrupt the reproductive potential of amphibians in these toxic oil sands regions.

Cypermethrin- and glyphosate-based pesticides: Synergy in tadpoles and antagonism in fish (PL)

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The herbicide glyphosate and the insecticide cypermethrin are key pesticides of modern management in soy and corn cultures. Although these pesticides are likely to co-occur in ephemeral ponds or aquatic systems supporting amphibian and fish wildlife, the toxicological interactions prevailing in mixtures of these two pesticides have seldom been studied. The current study evaluated the toxicity of equitoxic and non-equitoxic binary mixtures of glyphosate- and cypermethrin-based pesticides to the fish, *Cnesterodon decemmaculatus*, and to tadpoles of the common South American toad, *Rhinella arenarum*. In the experiments with tadpoles, two different combinations of commercial products were evaluated: Glyphosate Atanor® Cipermethrin Xiper25® and Glyphosate Glifoglex® Cypermethrin Glextrin®. Equitoxic and non-equitoxic mixtures were significantly synergic in both combinations of commercial products. The magnitude of the synergy was constant at around two-fold for all tested proportions of the Glyphosate Glifoglex® Cypermethrin Glextrin® mixture, whereas the magnitude of the synergy varied between 4 and 9 times in the Glyphosate Atanor® Cipermethrin Xiper25® mixture. Lethal concentrations 50 (LC_{50}) obtained in terms of toxic units (TU) for the equitoxic mixtures were 0.47 and 0.58 TU for the two combinations of products tested, respectively. For their part, experiments with fish were realized with mixtures of Glyphosate Glifoglex® Cypermethrin Glextrin® only. In this case, both equitoxic and non-equitoxic mixtures were significantly antagonistic, the magnitude of the antagonism varying between 1.56 and 7.36, depending on the proportions tested. The LC_{50} obtained for equitoxic mixture in fish was 1.52 TU. These results highlight the need to include both amphibian and mixture toxicity testing in the regulatory requirements for the registration of pesticide products.

Update on development of a standardized amphibian test method using *Lithobates* spp. (PL)

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Amphibians are sensitive indicators of environmental health; however, assessing the potential for effects on these organisms due to contaminants is complicated by a

limited toxicological dataset and a lack of standardized test methods, particularly for species that are relevant to Canadian environments. Environment Canada has sponsored research for standardization of an amphibian toxicity test method using *Lithobates pipiens* (leopard frog, formerly *Rana pipiens*) and *Lithobates sylvaticus* (wood frogs). Method development activities have included research on animal husbandry for all life-stages of these species; spawning, including application of hormones and dopamine antagonists to stimulate spawning; and toxicity test methodology. Methods for maintaining captive breeding colonies of amphibians are desirable to limit sampling stress on amphibian populations and increase standardization of test organisms. Test methods that have been investigated include 96-hour acute exposures and chronic tests that have been initiated using either egg or early tadpole stages, and involving exposure through to metamorphosis. Data gaps identified include those related to supply of test organisms, diet, disease prevention, test duration, test validity requirements and test termination criteria. Recommendations have been made for the steps that would be required to address these data gaps and finalize the test method as a standardized Environment Canada biological test method.

Arsenic speciation and AS3MT protein expression pattern in Western clawed frogs, *Silurana tropicalis* (PO)

Jing Zhang[1], Mark Button[1], Guilhem Caumette[1], Laura Gibson[1], Iris Koch[1], Kenneth Reimer[1], Valérie Langlois[1] and William Cullen[2]

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Arsenic exposure has raised concerns about public health and natural habitat of various species. Firstly, it is well documented that several health issues are related to arsenic administration, including many types of cancer. Arsenic toxicity is dependent on its speciation. It was suggested that inorganic arsenic species are more toxic than the organic forms, and that arsenite (AsIII) exhibited higher toxicity than arsenate (AsV) in living organisms. Once entering into the system, arsenic compounds undergo a series of enzymatic biotransformation steps, which produce a variety of methylated arsenic metabolites. The methylation-dependent arsenic metabolism uses S-adenosyl methionine (SAM) as the methyl group donor. The key enzyme driving this process is Arsenic (III) methyltransferase (AS3MT). Furthermore, in contrast to the extensive studies on arsenic-dependent human health issues, the knowledge of the impact of arsenic exposure on aquatic amphibians is fairly limited. The current study therefore addresses the topic by examining speciation and responses in protein level of AS3MT in the Western clawed frog (*Silurana tropicalis*) upon chronic aquatic arsenate exposure. Frogs were exposed from Nieuwkoop-Faber (NF) stage 12 to 66 to three inorganic arsenate treatments: 0, 0.5 and 1 $\mu g \cdot g^{-1}$. High-performance liquid chromatography coupled with inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) was used to measure arsenic speciation and biotransformation in frogs. The frog tissues contained tetramethylarsonium ion even at the earliest stage at which the arsenic could be quantified, NF 46, as well as at later

stages. No trimethylarsine oxide was present at stage NF46, but it was one of the dominant compounds at NF 56. Other arsenic species included inorganic arsenic, dimethylarsinic acid, arsenosugars, and even traces of arsenobetaine at NF 46, a point prior to the embryo's receipt of food. In addition, immunoblotting was used to measure the protein levels of AS3MT in NF stage 56 and 66 frogs. The results suggested stage-dependent protein levels. AS3MT exhibited a higher abundance in NF stage 66 frogs (whole body) than stage 56 tadpoles. Both NF stage 56 tadpoles and stage 66 frogs (whole body) showed changes in AS3MT protein level when compared between treatments. Overall, various arsenic species were detected in different developmental stages of the Western clawed frogs upon arsenate exposure; and the differential AS3MT expression profile under different NF stages and arsenic exposure intensities may play key roles in the observed arsenic biotransformation.

A refined effects determination for the California red-legged frog potentially exposed to malathion (PO)

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The California red-legged frog (CRLF) (*Rana aurora draytonii*) is endemic to California and Baja, Mexico. The species has been extirpated from 70% of its former range and was listed as threatened under the *Endangered Species Act* in 1996. The United States Environmental Protection Agency must determine whether 66 pesticides currently authorized for use in California may adversely affect the CRLF. A screening-level and refined effects determination (ED) was conducted for the CRLF to assess potential direct and indirect effects associated with exposure to the insecticide malathion. Our screening-level ED indicated that terrestrial-phase CRLFs are not at risk from direct exposure to malathion. Using more refined aquatic exposure models including the Soil and Water Assessment Tool (SWAT), three worst-case scenarios in California were modeled to estimate potential risk to aquatic-phase CRLF and their prey (fish and aquatic invertebrates). Such areas included natural ponds in the Watsonville slough core area, streams and rivers that drain the major agricultural regions of the Watsonville slough, as well as tributary drainage ditches with low flow, in which CRLF breeding may occur. To estimate the probability of direct and indirect effects to CRLF and their prey, distributions of estimated environmental concentrations (EEC) derived using the refined modelling were compared to concentration-response curves and species sensitivity distributions respective of the taxa of concern. The subsequent refined probabilistic ED indicated that malathion has a low probability of adversely affecting aquatic-phase CRLFs and their fish and invertebrate prey. The results of this ED indicate that risks of direct effects to CRLFs and indirect effects via reduction of prey and/or habitat, are negligible.

Identification of potentially toxic degradation products arising from sediment-borne exposures of Disperse Yellow 7 to *Silurana tropicalis* (PO)

Valérie Langlois[1], Vimal Balakrishnan[2], Justine Mathieu-Denoncourt[1], Shane de Solla[2] and Salma Shirin[2]

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Selected azo- and benzidine-containing dyes have been categorized as medium-priority compounds under the Government of Canada's Chemical Management Plan (CMP). Disperse Yellow 7 (DY7) is a disazo compound that is mainly used in textile industries and represents an important class of organic pollutants about which little information is known regarding their environmental fate. Organic compounds can undergo chemical and biological transformations when they interact with environmental matrices and biotic species; therefore, understanding these transformations is a critical aspect of assessing their environmental fate. While reduction processes are important in toxicology, the presence of biological tissues and sediments can often have a confounding effect in terms of identifying the products that would arise upon reductive transformation of a contaminant. Consequently, we used zero-valent iron to efficiently reduce DY7 to produce p-phenylenediamine (p-PDA), 4-aminoazobenzene (4-AAB), and 4-aminobiphenyl (4-ABP). These reaction products are known to be genotoxic (p-PDA) or carcinogenic (4-AAB, 4-ABP). Reaction products were identified using accurate masses obtained from high resolution mass spectrometry (LC/Q-ToF/MS) instrumentation. We also demonstrated that DY7 was toxic to *S. tropicalis* embryos, triggering mortality and malformations and increasing heat shock protein 70 and 90 mRNA levels, particularly at higher sediment exposure concentrations. When the sediment samples were analyzed by LC-MS/MS, we found that p-PDA, 4-AAB, and 4-ABP were all present in exposures, but not in any of the sediment controls. These results will be discussed in the context of their implications on possible environmental releases of DY7 in the aquatic ecosystems.

Assessing Wildlife Ecotoxicology

High sensitivity to dietary selenomethionine found in juvenile white sturgeon (*Acipenser transmontanus*) (PL)

Jenna Zee[1], Sarah Patterson[1], Steve Wiseman[1], Danielle Gagnon[1] and Markus Hecker[1]

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It has been shown that selenium (Se) released to the environment can have devastating effects on local aquatic wildlife. Of the various forms Se can take, selenomethionine (SeMet) is the most bioavailable and therefore the most toxic. For secondary consumers, ingestion is the most important method of Se uptake. Sturgeon are culturally important to First Nations and economically key to fisheries along the West coast of North America. However, many populations are in decline, experiencing poor recruitment and classified as endangered, the white sturgeon (*Acipenser transmontanus*) included. While the reasons for these declines are not yet fully understood, the sturgeon's benthic lifestyle, longevity, and position in the food chain leave them particularly susceptible to contaminant exposures. Current high concentrations of SeMet in diets of white sturgeon, with predicted increases in anthropogenic releases, have made it a contaminant of concern for this species. Therefore, this study was designed to investigate the sensitivity of juvenile white sturgeon to dietary SeMet. During a 72-day exposure white sturgeon were given feed containing 1, 5, 25 or 100 µg SeMet (dm) per g food. These doses corresponded to an uptake necessary for proper health, two environmentally relevant exposures, and a possible environmentally relevant exposure level in line with predicted releases of Se. Within 10 days of exposure, pathological effects were observed in the sturgeon given the high dose. Occurrence of severe edema, causing exophthalmos, developed within 15, 23 and 52 days in the high, medium and low doses respectively and lethal effects rates of 54% and 22% occurred in high and medium doses respectively. Tissue digestions indicated that Se accumulated in a dose-dependent manner and reached equilibrium in the high dose in approximately 40 days. Growth, liver weight and hepatosomatic index were all significantly lower in the high dose. Food avoidance was also observed in the high dose. These results indicate that white sturgeon are highly sensitive to the effects of Se accumulation over relatively short time periods. This stresses the need for continued sturgeon research and studies looking into the environmental fate and regulation of released Se.

Migration of fish-eating birds influences mercury concentrations (PL)

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Bird migration is thought to influence concentrations of contaminants in birds because of differential accumulation in spatially- and temporally-distinct ecosystems. We characterized (a) overwintering areas using stable isotopes of hydrogen (δ2H) and band recoveries; and, (b) overwintering habitats by combining information from stable isotopes of sulfur (δ34S), carbon (δ13C), nitrogen (δ15N) and δ2H in feathers grown during the winter. Two fish-eating bird species, the double-crested cormorant (*Phalacrocorax auritus*) and the Caspian tern (*Hydroprogne caspia*) breeding in Lake Ontario were chosen to measure the impact of long-distance migration on mercury concentrations [Hg]. Migration had a significant impact on [Hg]. High [Hg] were found for both species in habitats characterized by a food web supported by benthic or C4 primary production. Allochthonous import and *in situ* production of Hg (e.g., through sulfate reducing bacteria in sediments) would explain high [Hg] in birds. Birds overwintering in southernmost locations showed higher [Hg]. Mercury concentrations in winter feathers were related to predicted spatial pattern of [Hg] in fish based on bird spatial assignment (using δ2H). This study indicates that overwintering area greatly influences [Hg] and suggests that biologically-mediated transport of Hg occurs between wintering and breeding habitats.

Health of domestic mallards (*Anas platyrhynchos domestica*) following exposure to oil sands process-affected water (PL)

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Bitumen extraction from the oil sands of northern Alberta produces large volumes of process-affected water (OSPW) that contains substances toxic to wildlife, including residual bitumen, metals, polycyclic aromatic hydrocarbons, and naphthenic acids. Recent monitoring has shown that tens of thousands of birds land on OSPW ponds annually, creating an urgent need to understand its effects on bird health. In order to emulate the repeated short-term exposures to OSPW that migrating waterfowl are thought to experience in the oil sands, Pekin ducks (*Anas platyrhynchos domestica*) were repeatedly exposed to recycled OSPW. As indicators of health, a series of physiological (electrolytes, metabolites, enzymes, hormones, and blood cells) and toxicological (metals and minerals) variables was measured. Relative to controls, juvenile treatment birds had higher potassium following the final exposure, and juvenile males had a higher thyroid hormone ratio (T3/T4). Adult treatment birds had higher vanadium, and, following the final exposure, higher bicarbonate. Adult treatment females had higher bile acid, globulin, and molybdenum levels, and males, higher corticosterone. However, even for analytes that differed, means were within standard avian reference ranges, suggesting the absence of

significant toxicological or biological effects. While it is premature to assume that ponds containing recycled OSPW are not toxic to birds, these results suggest that recycled water ponds are substantially less dangerous than those containing bitumen and fresh tailings. Although more work is needed to determine the generality of these results, for ponds that are not acutely lethal to birds and do not elicit chronic or sublethal effects, current avian protection efforts might be relaxed. This change would permit higher protection, in the form of deterrent intensity, at the more toxic ponds. This scenario contrasts with current practices, which apply similar deterrent efforts across all types of OSPW ponds, potentially reducing, via habituation, bird protection from the constituents that are most likely to cause mortality.

Changes in thyroid function and hepatic status of American kestrels exposed to priority organophosphate flame retardants (PL)

Kim Fernie[1], Vince Palace[2], Lisa Peters[2], Nil Basu[3], Robert Letcher[1], Natalie Karouna-Renier[4], Sandy Schultz[4], Rebecca Lazarus[4] and Barnett Rattner[4]

[1]Environment Canada, [2]Stantec, [3]McGill University, [4]U.S. Geological Survey

Tris(2-butoxyethyl) phosphate (TBOEP), tris(2-chloroethyl) phosphate (TCEP), tris(2-chloroisopropyl) phosphate (TCIPP), and tris(1,2-dicholor-2-propyl) phosphate (TDCIPP) are priority organophosphate flame retardants (OPFRs) for risk assessment by Environment Canada and the U.S. Environmental Protection Agency. Detected in fish, avian and human tissues, their effects are poorly understood. Captive adult male American kestrels (*Falco sparverius*), exposed daily for 21 days to safflower oil vehicle (n=10), were compared to those exposed to one of these four OPFRs (n=7/OPFR) (2500 ng OPFR per 50 µL safflower oil per day). The OPFRs in exposed birds were more than likely depleted by enzyme-catalyzed transformation processes as they were generally not detectable in hepatic or renal tissues. At these realistic concentrations, no changes in body mass, hepatic oxidative stress, cholinesterase enzymatic activity, or nicotinic or muscarinic receptor levels were evident. Plasma clinical chemistry measures suggested modified hepatic integrity and function following exposure to TBOEP, TCIPP, and particularly TDCIPP. At 7 days, kestrels exposed to TBOEP had significantly elevated albumin:globulin (A:G) ratios and alkaline phosphatase activity. Kestrels exposed to TDCIPP had elevated bile acids (7 days), a likely mechanism of toxicity in chicken embryos exposed to TDCIPP, as well as lower A:G ratios (7, 21 days) contrary to TBOEP, and reduced alanine aminotransferase activity (14 days). Furthermore, exposure to all four OPFRs suppressed gamma-glutamyl transpeptidase enzymatic activity. Generally elevated levels of circulating thyroid hormones occurred at 7 days of exposure but were absent by 21 days, with the exception of reduced plasma thyroxine (T4) in TDCIPP-exposed birds. Hormones in circulation can fluctuate transiently under the influence of contaminant stress, but thyroid gland changes are perhaps more sensitive indicators of thyroid axis disruption. Changes in glandular structure and hepatic T4 deiodinase activity were evident at 21 days exposure. Decreased epithelial cell height and/or increased follicular

colloid suggested that kestrels exposed to TCEP, TCIPP or TDCIPP had less active thyroid glands. Elevated hepatic T4 deiodinase enzymes occurred with exposure to TBOEP, TCIPP and TDCIPP. Further research is required to further define the mechanism of action of OPFRs and identify possible effects on wild birds, given the greater concentrations of these OPFRs reported in their tissues.

European starlings (*Sturnus vulgaris*) as sentinels for health effects from urban air pollution in in Calgary, Alberta (PL)

Michelle North[1], David Kinniburgh[1], Mary Zhou[1] and Judit Smits[1]

[1]University of Calgary

Concern about the effects of chronic exposure to air pollutants in urban areas on human health sparked this investigation to identify suitable biomarkers of exposure in free-living wildlife. Wild birds cohabiting urban areas are ideal sentinel species for inhalation exposure of people, and are practical study subjects during their nestling phase. Concentrations of air pollutants (NO_2, SO_2, benzene, polycyclic aromatic hydrocarbons, and particulate matter) were determined by Health Canada in 2010/2011, dividing the city into low, medium or high pollution zones. We are verifying these categories using passive contaminant monitoring membranes placed at selected nest box sites during the breeding season. Pilot studies conducted during the summers of 2012 and 2013 sampled a total of 40 and 20 nestling European starlings respectively. Nest boxes placed around the city were monitored for reproductive success of the parents as well as survival, growth (weight, wing chord and tarsal length), immune (T cell) response (phytohamagglutinin skin test), and chronic stress (feather corticosterone) of the nestlings measured. Organ weights (liver, spleen, bursa of Fabricius) and histopathology of these organs were evaluated. These biological responses were analysed against air pollution levels in each location, for the individual pollutants as well as in combination, with the hatch date as covariate. Results demonstrated an increasing trend in corticosterone with increasing pollution, with NO_2 having the strongest effect. Additionally, measures of growth showed increased variability with increasing pollution. All other variables were not significantly associated with air contaminants. Results of the summer 2014 field season shall be discussed.

Impacts of lead and methylmercury on behaviour in chicken hatchlings (PL)

Theresa Johnston[1], Kimmo Mäenpää[1] and Nil Basu[1]

[1]McGill University

Lead (Pb) and methylmercury (Hg) are heavy metals widespread in the environment. Many types of birds are particularly susceptible to both metals, owing to dietary exposures and other factors. Though it is well established that both metals are

neurotoxic threats to birds, our understanding of their neurodevelopmental effects is still poor. In addition, though birds are often exposed to both metals in their natural environment, to our knowledge their combined effects have not been assessed. The objective of our study was to characterize the neurological effects (i.e., focus on neurochemistry, behaviour, and other physiological measures) of Pb, MeHg, and a combination of Pb/MeHg on fertilized chicken eggs (40 eggs per treatment) that were injected with lead nitrate (1-10,000 $ng \cdot g^{-1}$ egg) and MeHg (3000 $ng \cdot g^{-1}$ egg) at ecologically relevant concentrations. Mortality and developmental malformations were examined at hatching or during embryonic necropsy. Morphometric assessments of hatchlings were made 3 hours post-hatch. This was followed with behavioural assessments of birds at 5 and 10 days post-hatch. Malformations including cerebellar hypoplasia and incomplete closure of the navel were seen in embryos at high doses of Pb. Data also suggest that Pb limits heart rate response to temperature fluctuation. These data help support the notion that Pb can have damaging effects on population size and fitness of birds in the wild and that there is no "safe level" of Pb exposure. Earlier we had published on MeHg's neurological effects on hatchlings, and we are now conducting studies to investigate MeHg-Pb co-exposures. We will compare behavioural assessments to neurochemical differences to link the changes from Pb and MeHg exposure to organismal effects.

Exposure-related effects of polybrominated diphenyl ethers and emerging flame retardants: Insights from avian models (PL)

Jonathan Verreault[1] **and Kim Fernie**[2]

[1]Université du Québec à Montréal, [2]Environment Canada

Birds have been used extensively as sentinels of environmental contaminant exposure and ecosystem health. One contaminant class that has received notorious attention in recent years are the halogenated flame retardants added to consumer products (e.g., electronics/electric equipment, cars, upholstered furniture, etc.) to achieve fire safety standards. Growing evidence on the persistence, bioaccumulation, and toxicity of two polybrominated diphenyl ether (PBDE) mixtures led in 2009 to their inclusion in the Stockholm Convention Annex 1. The last PBDE mixture remaining on the market is the deca-BDE (composed of more than 97% of the fully brominated congener BDE-209), although a phase-out of this mixture was announced recently. A growing body of evidence suggests that the health of several bird species is adversely affected by the exposure to PBDEs, particularly those species occupying elevated trophic positions in the food web and/or breeding in urbanized and densely-populated regions. This presentation will give insights from recent case studies on effects of PBDEs and emerging flame retardants in both experimentally-dosed and naturally-exposed birds. More specifically, effects on incubation (incubation length, nest temperature), growth, thyroid hormone levels, courtship behaviours and reproductive success were demonstrated in a bird of prey, the American kestrel (*Falco sparverius*), dosed with penta-BDE mixture congeners. American kestrel pairs dosed with an emerging flame retardant (β-TBECH) also laid fewer

and lighter eggs, and exhibited poorer egg fertility than controls. Comparatively, urban-adapted ring-billed gulls (*Larus delawarensis*) exposed in the wild (Montreal area) to high concentrations of flame retardants (mainly deca-BDE) showed altered thyroid hormone ratios and bone (tarsus) mineral density (BMD).

Small mammal sentinels of health effects from oil and gas related pollution (PL)

Judit Smits[1] and Jaime Rodriguez-Estival[1]

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Canada's oil sands production has been marked by ongoing controversy about associated environmental impacts because of the potentially toxic compounds whose release and deposition onto soil, water and vegetation pose a conspicuous risk for surrounding ecosystems. Complex mixtures of pollutants include polycyclic aromatic hydrocarbons (PAHs) and metals such as lead (Pb), cadmium (Cd) and mercury (Hg), which are of specific concern due to their persistence, cumulative properties, and toxicity to vertebrates. Recent studies encompassing the Athabasca Oil Sands region observed increased deposition of total PAHs (mainly alkyl-PAHs) and metals in melted snow within 50 km of upgrading facilities, compared to more distant, undisturbed areas, suggesting these compounds are a consequence of oil sands activities. The purpose of our research was to determine how the reported PAHs and metals at environmentally relevant levels affect the health of local wildlife, using herbivorous small mammals as sentinel species. Our experimental study had four treatments; metals, PAHs, both metals and PAHs, and controls. Results from the experimental study followed by field studies on active oil sands sites will be presented. We discuss: (a) if PAHs and metals in food and water at environmentally relevant levels are bioavailable to small (herbivorous) mammals; (b) if these chemicals are absorbed and result in detectable tissue residues; (c) which tissues and organs are the best indicators of exposure; and (d) which are the most sensitive biochemical or hormonal responses to such levels of exposure.

Plant consumption by grizzly bears reduces biomagnification of salmon-derived polychlorinated biphenyls, polybrominated diphenyl ethers, and organochlorine pesticides (PL)

Jennie Christensen[1], Mark Yunker[2], Misty MacDuffee[3] and Peter Ross[4]

[1]Stantec, [2]Independent Consultant, [3]Raincoast Conservation Society, [4]Vancouver Aquarium

The present study characterizes the uptake and loss of persistent organic pollutants (POPs) in grizzly bears (*Ursus arctos horribilis*) by sampling and analyzing their terrestrial and marine foods and fecal material from a remote coastal watershed in British Columbia, Canada. The authors estimate that grizzly bears consume 341 to 1,120 mg of

polychlorinated biphenyls (PCBs) and 3.9 to 33 mg of polybrominated diphenyl ethers daily in the fall when they have access to an abundant supply of returning salmon. The authors also estimate that POP elimination by grizzly bears through defecation is very low following salmon consumption (typically less than 2% of intake) but surprisingly high following plant consumption (>100% for PCBs and organochlorine pesticides). Excretion of individual POPs is largely driven by a combination of fugacity (differences between bear and food concentrations) and the digestibility of the food. The results of the present study are substantiated by a principal components analysis, which also demonstrates a strong role for log K_{OW} in governing the excretion of different POPs in grizzly bears. Collectively, the present study's results reveal that grizzly bears experience a vegetation-associated drawdown of POPs previously acquired through the consumption of salmon, to such an extent that net biomagnification is reduced.

Nitrites and nitrates toxicity in viviparous fishes of the Central Plateau of Mexico (PO)

Rebeca Aneli Rueda-Jasso[1], Antonio Campos Mendoza[1] and Julio Orantes Avalos[1]

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The Lerma River, one of the most important in the country, is located in the western central part of Mexico. Its waters have allowed the development of farming and industry. From the second half of the 20th century, the environmental quality of the region has been significantly altered due to socioeconomic growth. Besides the overuse of water, the indiscriminate introduction of nutrients (NH_4 , NO_2^- and NO_3^-) through punctual (disposal of raw sewage) and diffuse contamination in water bodies is one of the main factors in environmental degradation and the loss of habitat. These alterations of the water have resulted in the diminution of the areas of distribution of various fish, and the shrinking or loss of their populations. Agriculture is the main economic activity in the Lerma Basin, and the use of agrochemical products has a great impact on water quality. In the Río Lerma meander (in La Piedad, Michoacán), the levels of nitrogen such as ammonium range from 1.4 to 3.78 mg $N \cdot L^{-1}$. Normally, aquatic organisms are adapted for relatively low levels of inorganic nitrogen. Because of this, abnormally high concentrations of NH_4, NO_2^- and NO_3^- negatively influence the ability of the fish to grow, breed, and survive. The Mexican norms indicate 25 and 15 $mg \cdot L^{-1}$ of total nitrogen to be daily and monthly maximum averages as thresholds for the wildlife. More stringent norms such as EPA-USA indicate tolerance levels of 0.5 and 1.27 $mg \cdot L^{-1}$ of total nitrogen in warm waters. The fish of the Goodeinae subfamily (endemic to the central plateau of Mexico) are one of several species affected by anthropogenic activities. Historical registers include eight species of this subfamily in the La Piedad Meander, of which only two can still be found today. This present study establishes the connection between the concentrations of nitrogenated compounds and their toxicity, and the decrease in the number of Goodeinae fish species. The results also show that only the most tolerant species can be found in the Lerma River, along with some exotic species. Therefore the necessity of joint

efforts by the authorities, users and stockholders is made clear in order to improve the norms and production practices, thus improving water quality and allowing for a better preservation of the species.

Potential effect of climate change on the bioaccumulation of mercury in two large-bodied fish species in northern Ontario (PO)

Alexandra Sumner[1], Tom Johnston[2] and John Gunn[3]

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Increasing temperatures in Canada's subarctic region are expected to alter many components of aquatic ecosystems, including the bioaccumulation of mercury in fish. It is important to understand how climate influences the concentration of this neurotoxin in fish in order to assess the future impacts that climate change might have on the safety of consuming wild fish in northern Ontario. To better understand how climate influences mercury bioaccumulation, patterns in fish mercury concentrations across a climatic gradient in Ontario were investigated. Two species of large-bodied fish, walleye and white sucker, have been sampled from 75 lakes throughout the Near and Far North of Ontario. These lakes are distributed over 9.0° of latitude and represent a range of climatic conditions (annual growing degree days 604-1599). Additionally, fish mercury concentrations are being analyzed with respect to chemical, physical and biological variables known to be influential, such as lake pH, dissolved organic carbon concentration, and fish trophic positions and growth rates. The results of this study will address important gaps in our current understanding of how climate affects fish mercury levels, and will be useful in assessing reference conditions in advance of further climate change.

Mercury biomagnification in the Chilean Patagonian marine foodweb: Preliminary results (PO)

Gustavo Chiang[1], Karen Kidd[1], Nelson O'Driscoll[2], Solange Jara[3], Mauricio Diaz-Jaramillo[4] and Kelly Munkittrick[1]

[1]University of New Brunswick / Canadian Rivers Institute, [2]Acadia University, [3]Universidad de Concepcion, [4]Universidad Nacional de Mar del Plata

Mercury (Hg) occurs naturally in the environment, but humans have altered its natural cycling by fossil fuel combustion and mining. Mercury is a global pollution problem due to its long-distance transport and toxicity, especially methylmercury (MeHg). There are many studies examining MeHg and its trophic transfer in Northern aquatic ecosystems; however, much less information is available for the southern hemisphere. Chilean Patagonia is a hotspot of biodiversity with unique geological (volcanos) and

physical (high orographic precipitation rate) characteristics in a less industrialized environment and an ideal area to study Hg transfer in the coastal food web. Melimoyu Bay (44°04'S, 72°51'W) is an isolated coastal environment within the influence of the Melimoyu volcano and the Marchant River whose headwaters are influenced by the glacier in the volcano. We assessed food web structure using stable isotope analysis (δ13C & δ15N) and fish stomach content. The samples were also analyzed for total (THg) and methylmercury (MeHg). Preliminary data show that benthic predatory fish muscles have higher THg (0.041-2.12 $\mu g \cdot g^{-1}$ dry weight (dw)) and MeHg (0.017-1.215 $\mu g \cdot g^{-1}$ dw) concentrations and forage in a very similar habitat (narrow δ13C values). Benthic invertebrates have low THg (0.0073-0.0766 $\mu g \cdot g^{-1}$ dw) and their MeHg concentration is highly variable (5-73% of THg). Regression models for the whole food web ($\log_{10}$MeHg or $\log_{10}$THg vs δ15N) show a higher positive slope for $\log_{10}$MeHg (R^2=0.837, b=0.258, $p<0.001$) than for $\log_{10}$THg (R^2=0.613, b=0.187, $p<0.001$). Ongoing work will include pelagic and near shore predatory fish and invertebrates to complete the foodweb structure and assess if carbon source has any impact on Hg biomagnification. These preliminary data show that the biomagnification process is similar in Patagonian marine food webs to that found in polar zones in the northern hemisphere and that there is a high potential to incorporate more Hg from possible atmospheric deposition.

Metal Mining

Toxicity profile of a mine tailings-dominated stream in a mixed land-use watershed in South Africa (PL)

Steve Kalule[1] and Stephen Klaine[2]

[1]North West University, [2]Clemson University / North West University

The landscape of South Africa is decorated with mines and associated mine tailings piles. In the past century the urban footprint has expanded to surround these mines. This has resulted in human developments adjacent to mine tailings-impacted waters. The Wonderfontein Spriuit (WFS) is an acid mine drainage (AMD) dominated stream just west of Johannesburg, South Africa. The watershed drained by this stream also contains suburban and agricultural land uses. Most importantly, the downstream impoundments serve as the intake for crop irrigation, livestock watering and drinking water for the city of Potchefstroom. As such there has emerged an intense interest in evaluating the health of the aquatic ecosystem and remediating any anthropogenic impacts. Surface water toxicity was evaluated throughout the watershed using *Daphnia magna* laboratory bioassays. Land use, AMD sources, and other discharges into the river were identified using visual inspection. Water chemistry varied throughout the watershed, with pH as low as 2.75 at upstream locations receiving raw AMD to more neutral pH values in the middle and lower reaches of the river. Dissolved uranium concentrations exceeded 250 $\mu g \cdot L^{-1}$ in the upper reaches of the stream but fell to below 20 $\mu g \cdot L^{-1}$ by the bottom of the watershed. Bioassays conducted on filtered, undiluted samples taken at selected sites along the length of river showed a significant reduction in both acute and chronic toxicities to *D. magna*. Approximately 55% of the variability in acute toxicity throughout the watershed could be explained by dissolved uranium concentrations alone. A significant reduction in toxicity was observed at sites located just downstream of wastewater treatment discharge into the river. Dissolved organic carbon (DOC) concentrations increased at sites downstream of the wastewater discharges into the river, and this resulted in significant reductions in both survival and reproduction of *D. magna*. These results suggest that stream segments downstream of wastewater treatment plant discharges were healthier and that the reason was the increase in pH and dissolved organic carbon in the stream. Future research will investigate the effects of pH and DOC on the toxicity of uranium to *D. magna* using a multifactorial test design. This work will provide valuable insight into the options for attenuation and treatment of AMD, and setting catchment water quality standards.

Can low micronutrient availability enhance sulfate toxicity to aquatic organisms? (PL)

Keith Pilgrim[1]

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The composition and relative proportions of dissolved macro-constituents, including sulfate, can have a significant effect on the toxicological expression of an individual constituent. Industrial process waters often have cation and anion balances that do not occur naturally. This can affect the outcome of standardized laboratory-based toxicity tests used to evaluate the potential toxicity of an industrial effluent. This presentation will focus on the process followed to identify micronutrient deficiency-mediated sulfate aquatic toxicity. Laboratory-based chronic toxicity tests with *Ceriodaphnia dubia* were conducted using water from a group of groundwater-filled mine pit waters located in the United States. This study concluded that sulfate was more toxic in mine pit water largely because of low concentrations of zinc, selenium (selenate), and natural organic matter.

Giant Mine: A decade-long complex case study (PL)

Tamara Darwish[1] **and Hilary Machtans**[1]

[1]Golder Associates

Fish and benthic invertebrates inhabiting the waters downstream of Giant Mine, an abandoned gold mine located in Yellowknife, NWT, are exposed to elevated levels of arsenic and other metals in both water and sediment. Slimy sculpin (*Cottus cognatus*) captured downstream of Giant Mine have consistently had higher arsenic tissue concentrations relative to sculpin captured in uncontaminated areas, indicating that the fish are being exposed to elevated levels of arsenic relative to the reference areas, and that the arsenic was in a bioavailable form. Differences in fish populations were observed; however, these differences were not consistent and were likely within the range of normal variation. Response patterns differ among populations and over time, irrespective of whether individuals originate from an arsenic-exposed or reference population. In addition to monitoring effects as a result of effluent discharge, a separate sediment study was conducted, which found that concentrations of arsenic in the sediment were elevated above applicable guidelines. Despite elevated arsenic concentrations in water, sediment, and in fish tissue, no obvious response in the health, survival or reproduction of the fish population was evident. Confounding factors, including intermittent effluent discharge, recreational use of the exposure area, slimy sculpin use as a monitoring species, and a complex mining history, make interpretation through the EEM program difficult.

Landforms are the key determinant of metal impact and restoration technology effectiveness (PL)

Shirley Neault[1] **and Steven Siciliano**[2]

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Moisture and erosional patterns dominate metal speciation distribution in impacted soils with relatively few differences in metal speciation across the landscape. In a non-managed setting, the highest organic matter quality occurs in depressions in the landscape, but these same positions also have the highest metal concentrations and greatest impacts on the microbial, invertebrate and plant community. Amendments that provide organic matter (i.e., compost) have better potential for improving conditions for plant survival and growth than amendments aimed at immobilizing metals (i.e., biochars). While liming and biochar amendments don't significantly increase plant growth, they do decrease plant uptake and hence the potential for food chain transfer. Based on our results, a landscape-delineated amendment strategy is needed in which highly eroded soils are managed to increase plant growth and landforms that collect moisture, managed to precipitate metals. Such a strategy will decrease metal transfer through the ecosystem and begin setting the ecosystem on a restoration trajectory.

Teasing out current effluent effects on benthos, using artificial substrates: a case study from Junction Creek (PL)

Bruce Kilgour[1] **and Allison Merla**[2]

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Junction Creek has been influenced for several decades by numerous urban, commercial and industrial inputs, as well as by current and historical metals released from various mine effluents or aerial emissions. Those confounding factors and historical impacts make it challenging to tease out the effects of today's metal mine effluents on fish and benthic invertebrate communities. Artificial substrates were recently used as part of an ongoing Environmental Effects Monitoring program for Junction Creek, to tease apart the two exposure pathways. Substrates colonized by "sensitive" benthos (i.e., from a clean reference river) were placed in Junction Creek both upstream and downstream of the regulated final effluent discharge points associated with three active operations mines. The study design included replication so that upstream/downstream differences could be statistically tested. The program also included assessment of benthos from the natural substrates. There was variation in the results among the three sites, with present-day effluent demonstrated to be potentially contributing to effects in two of three cases.

Complications of administering metal mining Environmental Effects Monitoring programs on historically-impacted environments (PL)

Kelly Wells[1] **and Cassandra Rees**[1]

[1]Canada North Environmental Services

In 2002, the Environmental Effects Monitoring (EEM) program was implemented as a science-based performance measurement tool to assess the adequacy of the *Metal Mining Effluent Regulations*, which apply to the current release of mine effluent. However, mining operations with a long history of treated effluent release or that have inherited historically contaminated sites prior to operation complicate the EEM decision process. Flexibility in the process is encouraged to ensure that resources are being effectively used to assess environmental effects caused by current effluent quality, and that ecosystem recovery from historic impacts can be monitored. This talk presents two case studies discussing these types of scenarios.

In the first case, an operating facility has been discharging treated effluent into the same drainage for 37 years. The near-field exposure area contains many confounding factors that make comparisons to reference areas challenging (i.e., head water, flow and water levels artificially elevated by sustained effluent release, and historical impacts due to past effluent quality). Effluent quality has changed dramatically over the course of the operation, including upgrades made over the past 7 years that have resulted in significant reductions of numerous metal concentrations in the surface water and sediment of the receiving environment. Considering the impacts associated with past effluent quality, the extended period of operation, the above-listed confounding factors, and the recent time frame of the treatment upgrades, the time period needed for recovery of benthic invertebrate communities to occur in the near-field exposure area, or what the endpoint of recovery will be, is uncertain. It is proposed that the most effective means of monitoring further recovery is to continue to assess temporal changes in both the physical environment and biota, as opposed to reference comparisons or additional investigation of cause studies.

In the second case, an operating facility is using a pre-existing Tailings Management Facility (TMF) that has been used for tailings storage since 1973 throughout various mining campaigns. To date, there has been no discharge of mine or tailings water by the current operation and all effluents released have consisted of freshwater diverted around the TMF and a few isolated dewatering events from the TMF. In 2013, the operation was required to conduct a Phase 1 EEM study and numerous EEM-defined effects were found between the near-field exposure area and multiple reference areas in both the benthic invertebrate and fish population endpoints. Water and sediment quality in the exposure area were very similar to baseline studies conducted before the current mine was operational, suggesting that effects are due to historical contamination. It would be ideal if the operation could use the study as a baseline EEM program to evaluate if effects change once mine or tailings water are discharged.

Comparison of Environmental Effects Monitoring requirements with existing monitoring requirements in the NWT: Is there any potential for harmonization? (PL)

Kathleen Racher[1] and Gord Macdonald[2]

[1]Wek'èezhìi Land and Water Board, [2]Diavik Diamond Mines Inc.

There are currently three diamond mines operating in the Northwest Territories (NWT) with one more slated for construction in the fall of 2014. Since the *Metal Mining Effluent Regulations* (MMER) does not currently apply to diamond mining, the sole regulators with respect to effluent limits and aquatic effects monitoring are the Land and Water Boards of the Mackenzie Valley. In the absence of the requirement to implement the Environmental Effects Monitoring (EEM) program as per the MMER, the NWT Boards have required proponents to design and implement aquatic effects monitoring programs (AEMPs) that entail, in most respects, more extensive monitoring, reporting and follow-up than the EEM. Based on the concerns of local stakeholders, the NWT Boards have also started requiring AEMPs of metal mines in the NWT, on the assumption that the EEM requirements could be harmonized to avoid unnecessary duplication of sampling, analysis or reporting by proponents. The ability to harmonize the EEM with the more extensive AEMP requirements will become even more important if the MMER is changed to include the regulation of diamond mines. In this talk, we will compare the typical requirements of a northern AEMP with the EEM and discuss any potential to harmonize the two types of programs.

Investigation of Cause at the Brunswick Mine: Relative contribution of physical and chemical factors towards ongoing biological effects (PL)

Paul LePage[1], Cynthia Russel[1], Pierre Stecko[1] and James Cormier[2]

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The Brunswick Mine, a massive sulphide zinc/lead mine located in northern New Brunswick, operated from 1964 to 2013. Historically, the mine discharged untreated effluent directly into the South Branch of the Little River, resulting in poor water quality throughout the Little River (very low pH events and high metal concentrations) and severely impacting the biology (including the elimination of fish from the South Branch). Lowest pH generally occurred well downstream of effluent discharge due to seasonal oxidation of thiosalts and other potential geochemical processes. Effluent treatment was improved over time starting with the construction of an effluent treatment plant in 1993 that ultimately included seasonal hydrogen peroxide injection to oxidize thiosalts (initiated in 2004). Since the initiation of treatment, chemical and biological recovery of the Little River has been remarkable, including temporal increases in benthic invertebrate density and taxon richness, and the re-establishment of fish populations. Despite the

substantial improvements, Environmental Effects Monitoring (EEM), initiated in 2005, identified effects to benthic invertebrates (fewer taxa and higher Bray-Curtis Index) and fish (reduced body condition of juvenile shite sucker) within the Little River.

The magnitude of the observed differences triggered Investigation of Cause (IOC). A total of four potential causes of the observed effects were identified: (a) effluent toxicity; (b) mine seepage; (c) river bed, bank and floodplain contamination; and (d) effluent discharge-related water level fluctuations. Effluent water chemistry investigations indicated that effluent discharge remains a dominant source of major ions and several metals, and still has some influence on river pH (which may be associated with oxidation and reduction of iron). Seepage and seasonal physical/chemical mobilization of historical contamination of river bed, banks, and floodplain represent sources of metals historically related to the mine (e.g., zinc and lead). Water level fluctuations associated with the intermittent operation of the effluent treatment plant have direct effects associated with low water (reduced habitat area), but also result in the exposure of creek substrate that, in turn, results in lower pH and metal release associated with sediment oxidation. Effects associated with physical habitat loss versus low pH/metal mobilization were indistinguishable in this study and may be the focus of future investigation. The current investigation highlighted the importance of oxidation/reduction in the release of metals from historically contaminated sediments.

Investigation of Cause of effects on benthic invertebrate communities downstream of two metal mines (PL)

Helga Sonnenberg[1] **and Lisa Ramilo**[1]

[1]Stantec

For the Investigation of Cause (IOC) study, an integrated assessment was designed to determine which metals were bioavailable and causing impacts on downstream benthic invertebrate communities and, whether effects observed were due to current receiving water quality or historical sediment contamination. Multiple lines of evidence were used, including extensive water and sediment chemistry; benthic community composition; toxicity testing using site sediment and receiving water carried out in the laboratory and in cages deployed in the field; and metals bioaccumulation in wild, field, and laboratory exposed *Hyalella*. Wild *Hyalella* were harvested, depurated and analyzed for metal content using ICP-MS. Five metals (Cd, Cu, Pb, Zn and Se) were found to be close to the laboratory-based Lethal Body Concentrations (LBCs) established in the literature; however, generally LBCs were not reached in wild organisms. Impacts on downstream benthic communities tested ranged from severe to moderate. The near field area (60-75% effluent) was devoid of *Hyalella* and the far field area was moderately impacted (30-40% effluent). Site water and sediment were collected from these areas and a reference area to conduct toxicity tests in the field and in the laboratory. *Hyalella* were used in a 3x3 factorial design using control sediment, reference site sediment and exposure/contaminated sediment, each with overlying water consisting of lab water,

reference site water or site exposure water (Environment Canada Test Method, EPS 1/RM/33, 1997). Upon termination of the toxicity tests, metals in *Hyalella* from all treatments were analyzed for metals. Laboratory toxicity tests indicated that both site collected receiving water and sediments from the near field area were toxic to *Hyalella azteca*. Comparison to LBCs indicated that copper in the creek water was sufficiently elevated to elicit chronic toxicity. In the far field area, neither the site receiving water nor the sediment appeared to be responsible for toxicity despite the fact that at this location, the abundance of wild *Hyalella* in benthic invertebrate communities was significantly reduced. Based on the metal concentrations in caged field *Hyalella* specimens, only zinc reached concentrations high enough to elicit chronic toxicity, and overlying water was also the predominant route of exposure. Based on site-specific chemical speciation modelling and the biotic ligand model (BLM), overall metal toxicity was predicted to decrease in the order of Cu > Zn > Cd; however, the new metal effects addition model (MEAM) (Norwood, 2013) provided additional insight regarding prediction of toxicity from exposure to mixtures of metals. Results of these models and field- versus lab-toxicity tests are compared.

Environmental Effects Monitoring data interpretation (PL)

David Huebert[1]

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The *Metal Mining Effluent Regulations* (MMER) came into force in Canada in June, 2002. One of the requirements within the MMER was the development of site-specific Environmental Effects Monitoring (EEM) programs. The EEM program requires the collection of a variety of data, including water and effluent chemistry, acute toxicity, sublethal toxicity, and biological information related to fish and benthic invertebrate communities. Over the past 12 years, all operating metal mines in Canada have collected the above-noted data in seeking compliance with MMER requirements, under the guidance of Environment Canada staff and the EEM guidance document. The implementation of the EEM program has therefore resulted in the accumulation of a considerable amount of water- and effluent-chemistry data, toxicological data, and biological data from rivers, streams and lakes across the country. Further, the data have been collected using relatively comparable methods and, generally speaking, with the oversight of Environment Canada regional staff. The Mining Association of Canada has sought to undertake an assessment of the accumulated raw EEM data independent of Environment Canada. The objective was to determine the prevalence of “effects” within the meaning of the EEM, and to develop an understanding of the correlation between EEM chemical, toxicological, and biological data. It was determined that prevalence of “effects” was reduced by 50% when using a Critical Effect Size (CES) to evaluate the data, and a further 10% when using an adjusted p-value. These results suggest that inclusion of CES is of critical importance in determination of the environmental significance of observed “effects” within the metal mining industry. Further, there was no observed

correlation between *Daphnia magna* acute lethality and effluent chemistry or biological effects. These results suggest that acute lethality testing does not provide useful regulatory information related to metal mining discharge.

MEND BATEA study (PL)

Kristin Pouw[1], **Kathryn Campbell**[1] **and Lisa Babel**[1]

[1]Hatch

Hatch was commissioned by the Mine Environment Neutral Drainage (MEND) Program to complete a study to identify best available technologies economically achievable (BATEA) to manage and control effluent from mines in Canada. The study was commissioned in order to provide reference information to policy makers, industry, and civil society organizations for use in evaluating potential forthcoming changes within the *Metal Mining Effluent Regulations* to the types of regulated mining facilities, the list of Schedule 4 parameters, and the authorized limits of Schedule 4 concentrations in effluent discharged to the environment (outlined in the 2012 Environment Canada discussion paper, "10-Year review of *Metal Mining Effluent Regulations*"). This paper presents the overall objectives and methodology of the study and the participatory process used to gather and validate information, an overview of the various Canadian mining subsectors examined (metal mining: base metal, precious metal, iron ore, and uranium; diamond mining, and coal mining), a summary-level review of technologies considered to be best available technologies (BAT) for Canadian mine effluent treatment, and incremental costs of implementing and operating BAT. BAT are defined as those technologies which have been demonstrated to achieve the present MMER Schedule 4 limits via treatment of mine effluent under representative Canadian climate conditions. Finally, the paper presents Hatch's findings on best available technologies economically achievable (BATEA)—that is, those technologies that serve to improve effluent quality for a given subsector within reasonable incremental capital and operating costs, based on best professional judgment. The study largely focuses on existing operations; however, some BATEA suggestions for greenfield operations are also made.

Towards Sustainable Mining (PL)

Ben Chalmers[1]

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The Towards Sustainable Mining (TSM) initiative is the Mining Association of Canada's (MAC) commitment to responsible mining. Participation in the program is mandatory for all MAC members. It is a set of tools and indicators to drive performance and ensure that MAC members are doing the right things for environmental protection and operational excellence at their mine sites. To measure performance, management

practices are assessed against indicators for six protocols: tailings management, biodiversity conservation, energy use and greenhouse gas emissions management, Aboriginal and community outreach, crisis preparedness, and safety and health. This presentation provides an overview of the TSM initiative which describes the environmental management components and how they are applied at mining facilities across Canada and abroad. It also includes results from the 2014 progress report. TSM is the only mining-specific standard that includes annual public reporting of facility-level results and independent verification.

Establishing dose-dependent developmental effects of maternal dietary selenium exposure in the model amphibian, *Xenopus laevis* (PL)

Anita Masse[1], David Janz[1] and Jorgelina Muscatello[2]

[1]University of Saskatchewan, [2]Stantec

Selenium (Se) is a contaminant of potential concern in aquatic systems located downstream of mining operations due to its efficient incorporation into food webs, potential for bioaccumulation at higher trophic levels, and role as a developmental toxicant in oviparous vertebrates. Adverse reproductive effects of Se have been the primary focus of research in fish and bird species; however, studies focusing on Se toxicity in amphibians are lacking, particularly early life stage toxicities and tissue-based toxicity thresholds. The objective of this study was to determine dose-response relationships for early life stage toxicities in the model amphibian, *Xenopus laevis*. Following a 68-day dietary exposure to food augmented with L-selenomethionine at measured concentrations of 0.7 (control), 10.9, 30.4, and 94.2 µg $Se \cdot g^{-1}$ dry mass, adult female *X. laevis* were bred with untreated males. Evaluations in the resulting eggs/embryos included Se concentrations, fertilization success, hatchability, survival, developmental rates, and the frequency and severity of Se-induced malformations. At the time of breeding, survival, hepatosomatic index, and gonadosomatic index of adult female *X. laevis* after exposure to dietary Se exposure showed no significant differences among treatment groups. However, a trend for increased female weight loss was observed with increasing dietary Se concentrations over the 68 days. Percent hatchability of embryos and survival of embryos/tadpoles up to 5 days post-fertilization (dpf) were significantly reduced in the 30.4 µg $Se \cdot g^{-1}$ treatment group compared to the control group. Cumulative mortality in tadpoles from 5 dpf to completion of metamorphosis showed no significant differences among treatment groups. There were no significant differences in the time to 50% metamorphosis among treatment groups. Further research will determine egg Se concentrations, fertilization success, and the frequency/severity of malformations in 5 dpf tadpoles. In addition, glutathione peroxidase activity, reduced/oxidized glutathione concentrations, and lipid peroxidation will be determined in tadpoles to evaluate potential effects on antioxidant defense mechanisms and their relation to Se-induced malformations. Overall, this study aims to aid in further understanding the sensitivity of

amphibians to Se, with the goal of developing environmentally relevant toxicity thresholds.

Integrative assessment of selenium speciation, biogeochemistry and distribution in a northern coldwater ecosystem (PL)

David Janz[1], Karsten Liber[1], Ingrid Pickering[1], Cheryl Wiramanaden[2], Shari Weech[2], Maria Gallego-Gallegos[1], Melissa Driessnack[1], Eric Franz[3], Meghan Goertzen[4], James Phibbs[1], Justin Tse[1], Kevin Himbeault[5], Erin Robertson[6], Charlene Burnett-Seidel[5], Kent England[5] and Anne Gent[5]

[1]University of Saskatchewan, [2]Minnow Environmental, [3]Azimuth Group, [4]Lorax Environmental, [5]Cameco Corporation, [6]K+S Potash

The aquatic ecotoxicology of selenium (Se) in coldwater ecosystems has emerged as an important concern in many regions of the world. For the past decade, extensive research has been conducted at a series of small lakes receiving treated liquid effluent containing elevated Se from a uranium milling operation in northern Saskatchewan, Canada. An underlying focus of these studies was to determine Se speciation profiles (using X-ray absorption spectroscopy) in the major biotic and abiotic compartments of these lakes. The aim of this presentation is to compile the site-specific information obtained from this research into an integrative perspective regarding the influence of Se speciation on biogeochemical cycling and food web transfer of Se in coldwater ecosystems.

Selenium concentrations in sediments displayed great spatial heterogeneity that was strongly correlated with percent total organic carbon (%TOC) content in a given sediment sample. On average, approximately 50% of sediment Se was in the form of elemental Se, although this ranged from 0 to 81% among samples, which showed positive correlations with finer particles (less sand) and %TOC content in sediments. Other Se species detected in sediments included selenosulfides, selenite, and inorganic metal selenides. In contrast, the major Se form in sediment-associated biofilm/periphyton was an organoselenium species modelled as selenomethionine (SeMet), illustrating the critical importance of this matrix in biotransformation of inorganic Se to organoselenium compounds and subsequent trophic transfer to benthic invertebrates at the base of the food web. Detritus displayed a Se speciation profile intermediate between sediment and biofilm, with both elemental Se and SeMet present. In benthic detritivore (chironomid) larvae and emergent adults, and in foraging and predatory fishes, SeMet was the dominant Se species. The proportion of total Se present as a SeMet-like species displayed a direct nonlinear relationship with increasing whole-body Se in invertebrates and fishes, plateauing at approximately 70-80% of total Se as a SeMet-like species. In fish collected from reference lakes, a selenocystine-like species was the major Se species detected. Similar Se speciation profiles were observed using 21-day mesocosm and *in situ* caging studies with native small-bodied fishes, illustrating the efficient bioaccumulation of Se and utility of these semi-controlled approaches for future research. Collectively, these

studies demonstrate the important role of speciation in governing food web assimilation and trophic transfer of Se in this coldwater aquatic system. The perspectives and considerations presented here are likely applicable to a wide range of northern industrial sites receiving elevated Se loading into aquatic ecosystems.

Proposal for a Canadian aquatic life guideline for selenium (PL)

Guy Gilron[1], David DeForest[2], Sarah Hughes[3], Kevin Brix[4], James Elphick[5], Carrie Rickwood[6], Adrian deBruyn[7], Lucinda Tear[2], William Adams[8] and Erin Robertson[9]

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There is now general consensus that selenium (Se) concentrations in fish tissue, especially eggs or ovaries, are most appropriate for evaluating whether Se concentrations in aquatic systems may pose unacceptable risks to aquatic species. However, there is still often a need to translate a fish tissue-based value to a water-based value on a site-specific basis, since water Se concentrations can be more readily monitored and regulated. We first developed a freshwater Se guideline for Canada based on concentrations in fish eggs/ovaries by applying the Canadian Council of Ministers of the Environment (CCME) protocol for deriving guideline values. When sufficient toxicity data are available, as there are for Se in fish eggs/ovaries, the CCME protocol recommends deriving guidelines as the 5th percentile of the species sensitivity distribution (SSD). For most fish species, Se EC_{10} values (i.e., 10% reproductive effect concentrations) were available or could be derived, whereas for some species, only no-observed-effect concentrations and/or lowest-observed-effect concentrations were identified. The 5th percentile egg/ovary Se concentration from the SSD was derived to be 20 $\mu g \cdot g^{-1}$ dry weight (dw), using the best-fitting distribution; this was considered a conservative and broadly-applicable guideline. The second step was to translate the fish egg/ovary-based Se value to water Se screening values that, when not exceeded, would result in a low likelihood that mean fish egg/ovary Se concentrations at a site would exceed 20 $\mu g \cdot g^{-1}$ dw. A multi-step modelling approach was applied, which consisted of the use of enrichment factors, invertebrate trophic transfer factors (TTFs), and fish TTFs, to translate the fish tissue Se guideline value back to possible surface water Se screening guidelines. Original Se bioconcentration and trophic transfer studies were conducted in order to augment existing datasets. Quantile regression analysis at each step of the multi-step model was used to derive generic water Se screening guidelines. The final values were determined to be 5.4 and 2.1 $\mu g \cdot L^{-1}$ for lotic and lentic water bodies, respectively. In addition, a sulphate-dependent water Se screening guideline was derived for selenate-dependent waters, as increasing sulphate concentrations reduce the bioavailability of selenate in aquatic food webs. We believe that these proposed fish egg/ovary and water screening guidelines reflect the current state-of-the-science regarding Se fate and effects, and are defensible values for interpreting and regulating Se concentrations in freshwater systems in Canada.

Inter-laboratory comparison reveals critical issues with periphyton community assessment (PL)

Shari Weech[1], Patti Orr[1], Michael White[1] and Carla Fraser[2]

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Analysis of periphyton is routinely requested by some Canadian regulators as part of aquatic effects monitoring programs for mines. Provincial technical guidance exists for sample collection; however, the guidance is not tightly prescribed and there are information gaps related to methods for collection and analysis of samples and the endpoints to be reported. To assess the current state of the science with respect to laboratory analysis, seven periphyton samples were homogenized, split in quarters and sent to each of four commercial laboratories for taxonomic identification and enumeration. Even after standardizing for differences in algal taxonomy reported by each of the labs, results indicated substantial differences in community composition in each sample. Rarely was the same species identified by all four laboratories in the same sample. The proportions of species identified by one laboratory that were also identified in the same sample by at least one other laboratory never exceeded 58%, meaning at least 42% of all species identified in a given sample were unique to a specific laboratory. Even when the data were collapsed to genus level, there was very little agreement among laboratories. In addition to the differences in taxon identifications, there were substantial differences in reported organism densities, indicating that absolute or relative taxon densities reported by different laboratories cannot be reliably compared.

Two overarching issues were identified that currently undermine the utility of periphyton community as an aquatic environmental monitoring tool: (a) lack of standardized methods for laboratory sample handling, analysis and QA/QC; and (b) no formal program for independent verification of taxonomic identifications (e.g., a program for taxonomist certification and/or laboratory performance testing). Despite these issues, regulators in some Canadian jurisdictions are increasingly requiring industries to incorporate periphyton monitoring in aquatic baseline and operational monitoring programs. Consequently, the demand for periphyton taxonomy services has increased, and the limited number of commercial laboratories available have become extremely back-logged (i.e., delays of up to five months between delivery of samples to the laboratory and reporting of results, or even refusal to accept new samples). With so little agreement of results among the four laboratories tested, it was not possible to infer which of the laboratories, or associated procedures, provided the most accurate results. Clearly, much additional research will be required by regulators and commercial laboratories toward standardization of laboratory methods and certification programs before periphyton community monitoring can be confidently incorporated into aquatic effects monitoring programs.

Determining the effect of a reduced YCT diet on the *Ceriodaphnia dubia* sublethal toxicity test in soft water in relation to the *Metal Mining Effluent Regulations'* Environmental Effects Monitoring program (PO)

Morgan King[1] and Carrie Rickwood[1]

[1]Natural Resources Canada

Research to develop predictive models for metal toxicity have highlighted that the food required to conduct sublethal tests can greatly reduce metal and effluent toxicity. This is especially true for metals with a binding affinity for organic matter such as copper (Cu). The *Ceriodaphnia dubia* reproduction test is a required sublethal toxicity test under the *Metal Mining Effluent Regulations*' Environmental Effects Monitoring program. The test uses a diet composed of equal amounts of YCT (yeast, cerophyll and trout chow) and algae. The YCT, a source of organic matter, has been proven to interfere with the toxicity of specific elements during these tests (Rickwood 2010). It was also determined that a reduced YCT diet in hard water resulted in greater sensitivity of organisms to Cu but not to zinc (Zn), which may be due to water hardness. Further toxicity testing with Zn in soft water was undertaken to eliminate any protective or potentially confounding effect of hardness on Zn. Therefore, our goal was to conduct side-by-side *C. dubia* tests in both hard and soft water, with and without manipulation of the feeding regime for both Cu and Zn. In addition, side-by-side toxicity tests with both Cu- and Zn-based effluent (diluted with hard and soft water) were performed using the normal feeding regime and alternate diets with reduced YCT and algae-only. Cu toxicity results were altered when YCT was reduced from the *C. dubia*'s diet, both in hard and soft water tests. More sensitive EC_{50} and IC_{25} were found when YCT was significantly reduced from the culture's diet, indicating that organic matter may have had an effect on the toxicity of Cu. However, the reverse was true for *C. dubia* exposed to Cu-based effluent; the alternative diet had no effect on Cu toxicity. For Zn exposures, soft water was the defining source of increased toxicity and sensitivity. Diet played little to no role in altering toxicity. However, *C. dubia* sensitivity to Zn-based effluent was not affected by water hardness or an alternative diet.

Thorium and radium toxicity in the aquatic environment (PO)

Nathalie Paquet[1], Christian Bastien[1], Nathalie Dassylva[1], Nicolas Gruyer[1] and Gaëlle Triffault-Bouchet[1]

[1]Ministère du Développement durable, de l'Environnement et de la Lutte contre les changements climatiques

The north of the province of Quebec has many uranium reserves, and the Quebec government is currently studying the possibility of allowing the exploitation of this resource. Although the exploitation of uranium ore will be governed by different guidelines and requirements, residual concentrations could be found in the surrounding

environment. In addition to uranium, its main descendants, thorium and radium, should be released in the environment. Since uranium has never been exploited in Quebec and the aquatic toxicity of thorium and radium is not well known, the exploitation of uranium will require the establishment of specific criteria. The goal of this project is to assess the toxicity of thorium and radium at low concentrations in realistic aquatic media. The concentrations of radionuclide were chosen to simulate the increase in the surrounding environment of mining sites. Standardized toxicity tests were performed. The tests included green alga growth inhibition using *Pseudokirchneriella subcapitata*, reproduction inhibition and lethal toxicity with *Dapnia magna*, growth inhibition of the freshwater macrophyte, *Lemna minor*, and lethal toxicity using rainbow trout (*Oncorhynchus mykiss*). The tests were carried out in treated tapwater and in an oligotrophic water, to represent the conditions of typical ecosystems of northern environments. This poster presents the first results of this project.

Application of the reference condition approach and test site analysis in the Porcupine River watershed (PO)

Saloni Clerk[1] **and Maggie Neff**[1]

[1]Ontario Ministry of the Environment and Climate Change

The Porcupine River near Timmins, Ontario, runs through a region with a long history of mineral extraction activities dating back to 1909. A major gold field is located within the watershed and a number of gold mines are located upstream of Porcupine Lake. Presently, there are only a few active mines, but historically, numerous mines were in operation. Assessing potential impacts to benthic communities is challenging in this watershed as pre-disturbance data is lacking and upstream reference sites do not exit. To assess whether benthic communities have been impacted by mining activities, the Environmental and Monitoring Branch of the Ontario Ministry of the Environment conducted a biomonitoring investigation of the Upper Porcupine River in 2011. Based on data collected, we assessed benthic invertebrate community metrics as well as sediment and water quality parameters. To assess potential impacts to benthic communities, the Reference Condition Approach (RCA) and Test Site Analysis (TSA) were applied to 13 sites in the watershed. The Porcupine River Watershed is an ideal system for application of the RCA and TSA given the lack of historical monitoring data. RCA and TSA results are compared to measured sediment and water quality, and priority areas for source control measures are identified.

Moving beyond Toxicity and Use Protection in Water Quality Management

This interactive session was a panel discussion, which began with each panel member presenting a brief platform presentation. Session chair, Dr. Neil Hutchinson (Hutchinson Environmental Sciences), then presented some questions to the panel, and invited comments and questions from the audience. Dr. Hutchinson was the moderator, and Richard Nesbitt (Hutchinson Environmental Sciences) served as rapporteur. Abstracts are not available.

Regulatory perspective: How to write water licences for no substantial alteration (PL)

Kathleen Racher[1]

[1]Wek'èezhìi Land and Water Board

Legal perspective: History of land claims agreements and why water quality is enshrined in them (PL)

Charles Birchall[1]

[1]Willms & Shier Environmental Lawyers LLP

Water quality as a stand-alone Valued Ecosystem Component in environmental assessment: Example from Kitikmeot Region

Richard Nesbitt[1]

[1]Hutchinson Environmental Sciences

Case study: Water quality as a Valued Ecosystem Component supporting protection of fish in environmental assessment

Tasha Hall[1]

[1]Golder Associates

Omics Science in Support of Effects-based Monitoring

Transcriptional responses of zebrafish to pharmaceutical and wastewater exposure: Are single compound exposures predictive of mixtures? (PL)

Joanna Wilson[1], Andrew McArthur[2], Emily Smith[1], Michal Galus[1], Sarah Higgins[1], Nina Kirischian[1] and Judy Jeyaranjaan[1]

[1]McMaster University, [2]Andrew McArthur Consulting

Human pharmaceuticals have been well documented in receiving waters, yet their impacts on aquatic species are not clear. We have exposed adult zebrafish (*Danio rerio*) for 6 weeks to waterborne acetaminophen, gemfibrozil, venlafaxine, and carbamazepine at two doses (0.5 and 10 $\mu g \cdot L^{-1}$). Fish were then exposed to a mixture of all four pharmaceuticals or wastewater effluent (5 and 25%) to assess whether transcriptional responses are similar with mixtures. For all exposures, reproduction was significantly reduced and histopathological changes were induced in kidney with at least the high dose exposure. Livers were pooled to provide sufficient RNA for microarray analyses. Hepatic transcriptional responses were determined with a modified Agilent 44K zebrafish microarry using a single channel approach. Significantly different probes were identified with 2-way ANOVA (sex and treatment) and rank product analyses with a 10% false discovery rate. Transcriptional responses were particularly marked with acetaminophen exposure and there was broad overlap in the significant probes found between doses and across gender for this compound. 52 probes were at least 20-fold up- or down-regulated in acetaminophen-exposed fish; 3 probes were 100-fold up-regulated (apolipoprotein Eb precursor, cdc73, and a hypothetical protein). Unique probes were identified for all exposures, suggesting that a unique transcriptional response may occur for each pharmaceutical, the pharmaceutical mixture, and wastewater effluent. Interestingly, there was almost no overlap in the transcriptional response found with single pharmaceutical exposure and either the mixture or wastewater effluent exposure. Indeed, the large transcriptional response from acetaminophen exposure was largely absent in fish exposed to the pharmaceutical mixture and wastewater effluent. This suggests that identifying individual or clusters of genes that may be useful in effects-based monitoring may be difficult for pharmaceutical compounds. (NSERC STPSC357050).

Waterborne fluoxetine exposure induces dose and sex-depended changes in hepatic microRNAs in *Carassius auratus* (PL)

Brooke Cameron[1], Paul Craig[2], Thomas Moon[1] and Vance Trudeau[1]

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MicroRNAs (miRNAs) are small non-coding RNAs that negatively regulate mRNA abundance and translation and contribute to the epigenetic control of gene expression. Hormones and endocrine-disrupting chemicals can regulate physiological processes by altering miRNA production. Fluoxetine (FLX), a selective serotonin re-uptake inhibitor (SSRI) and the active ingredient in Prozac, is found in the environment and disrupts feeding, stress, and reproduction in fish by acting on SERT, the serotonin reuptake transporter protein in the brain and liver. Male and female goldfish were exposed to environmentally relevant levels of FLX (0, 0.5, and 1 $\mu g \cdot L^{-1}$) for 7 days, and six previously identified FLX-targeted miRNAs in the liver were investigated. These miRNAs, dre-let-7d, dre-miR-22b, dre-miR-140-5p, dre-miR-210-5p, dre-miR-301a and dre-miR-457b are associated with the negative regulation of anabolic metabolism pathways in zebrafish (*Danio rerio*). These pathways include adipogenesis, cholesterol biosynthesis, triacylglycerol synthesis, and insulin signaling. These miRNAs were chosen due to the close phylogenetic relationship of goldfish and zebrafish. Following a week-long waterborne exposure to FLX, all six miRNAs were significantly increased in female goldfish liver ($p<0.05$) at the highest FLX treatment, while males showed no significant difference, indicating a dose and sex-specific response. These findings contribute to a better understanding of the mechanism of action of FLX on goldfish liver metabolism. While the miRNA sequences are well conserved across species, their mRNA targets may be less conserved and therefore the specific mRNA sequence targets in goldfish remain to be identified. [Funded by Natural Sciences and Engineering Research Council of Canada, Ontario Graduate Scholarship and the University of Ottawa Research Chair Program.]

Merging DNA damage with gene expression analysis: Towards a genotoxicogenomic approach (PL)

Francois Gagné[1] and Chantale André[1]

[1]Enviroment Canada

The effects of contaminants on the genetic material are well-known where the formation of DNA adducts and damage are often used to determine the genotoxic potential of various chemicals and determine DNA damage and repair in wildlife. The formation of DNA adducts either directly or through xenobiotic-induced reactive oxygen species leads to the formation of abasic sites and DNA strand breaks. DNA strand breaks are determined by measuring the amount of single- and double-stranded breaks in tissues. Sustained damage of genomic DNA increases the likelihood of repair errors leading to heritable gene alterations that could challenge organisms' survival, growth and

reproductive success. The evaluation of genes and their expression in damaged DNA could provide valuable insights into the long-term health risk in exposed organisms to genotoxic agents. This could also provide insights into the influence of damaged DNA and repair with respect to gene expression in organisms at the fundamental level. For example, DNA strand breaks containing genes involved in apoptosis could lead to altered apoptosis signalization in cells, which could favour the proliferation of altered cells. Current experiments are underway to determine the presence of damaged genes involved in endocrine regulation of gametogenesis (reproduction) with respect to their expression (mRNA) in exposed fish cells. An attempt is made to examine the interplay between the number of genes found in DNA double-stranded breaks and their expression in cells. This work will establish the basis of an environmental genotoxicogenomic approach to evaluate the risk of xenobiotics in aquatic organisms.

Variability in transcriptomics: Considerations for environmental applications (PL)

Chris Martyniuk[1], Andrew Cowie[2], Rick Wood[2], Yasmin Chishti[1], Joseph Mudge[2], Jennifer Loughery[2] and Jeff Houlahan[1]

[1]University of Florida, [2]University of New Brunswick

Transcriptomics is increasingly used in both the laboratory and field to assess biological responses to stressors such as aquatic pollutants. However, fundamental studies characterizing individual variability in transcripts are lacking, which currently limits the use of transcriptomics in environmental monitoring assessments. We addressed variability in the transcriptome of the fathead minnow (*Pimephales promelas*) by investigating 231 microarrays, which allows for repeated measures for the same gene probe. We found that some gene networks showed high variability (based upon the variation in expression of individual members within the network), such as cell proliferation, metabolism (steroid, lipids, and glucose), and vascularization, while others showed low variability (more stability), including mRNA and rRNA processing, regulation of translational fidelity, and ribosome biogenesis. We also measured the transcript abundance of 18 enzymes in the steroidogenic pathway as well as sex-steroid receptors in FHM ovary in order to quantify individual variability. We found that estrogen receptor 2b (esr2b), membrane progestin receptors (beta and gamma), and 5α-reductase a2 and a3 (srd5a2 and srd5a3) showed the highest variability in the ovary (CV=~1.2-3.0) while progesterone receptor (pgr), androgen receptor (ar), and, esr2a showed low variability in the ovary (CV=~0.5-0.7). Sample size estimates for real-time PCR experiments showed that 7 of the 18 transcripts (~40%) would require a sample size of greater than 20 in order to detect a two-fold change with sufficient experimental power (0.8). The variability of these transcripts will depend upon many factors, including breeding strategy of the species. For example, a meta-analysis of the literature revealed that 3β-hsd mRNA levels were more variable in single spawning fish compared to multiple spawning fish, while esr1 was less variable in single spawning female fish. These studies address some

important issues for ecotoxicology, namely what the target critical effect sizes for molecular data are, how to better standardize transcriptomics data for comparisons, and how to decide whether a gene is altered or not in a complex environment. Moreover, knowledge of variability in transcript levels will assist in determining an optimal significance level that minimizes both false positive and false negative errors in the interpretation of differential expression data in field studies.

Metabolomics as a tool for effects-based monitoring: Challenges and potential opportunities (PL)

Jonathan Benskin[1], **John Cosgrove**[1] **and Susie Huang**[1]

[1]AXYS Analytical Services Ltd.

Conventional assessments of wildlife health have relied on observations of individual vigor or gross measurements of population incidences of morbidity or mortality. In the event of moderate or severe contaminant impact, these measures will inevitably be "after the fact" and may be too late to enable timely remediation. The emerging discipline of "metabolomics" may offer more sensitive and discrete measurement of contaminant challenge and, therefore, earlier opportunity for remedial interventions. In the present work, we report on the development of a targeted metabolomics platform for quantification of over 200 metabolites, including acylcarnitines, amino acids, glycerophospholipids, ∑hexose, sphingolipids, and biogenic amines, fatty acids, and bile acids by flow injection- or liquid chromatography-tandem mass spectrometry (FI-MS/MS or LC-MS/MS, respectively). The application of the method to lab- and field-based exposures involving a diverse range of aquatic species and complex environmental samples will be discussed, including potential applications for "barcoding" contaminant exposure in a sentinel species.

Don't panic: The hitchhiker's guide to 'omics methods in non-model species (PL)

Caren Helbing[1]

[1]University of Victoria

With a wide range of omics-based methodologies available and the promise of mechanistic and predictive information relevant to effects-based monitoring, the potential for use is exciting and mind-boggling. Most available techniques have been established in model organisms, many of which are not necessarily relevant for the environments that require the monitoring and protection. This has largely driven what is available through largely medically-oriented models. How can we change this? How feasible is it to apply omics methods to non-model species that would be more informative for effects-based monitoring? Bring your towel, as this presentation provides

examples of how a variety of high-powered omics techniques can be applied to a wide range of ecologically-relevant non-model species in the laboratory and in the field.

The use of plasma proteomics in support of effects-based monitoring: What we have learned from field studies (PL)

Denina B.D. Simmons[1], Bernard Duncker[2] and James Sherry[3]

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For effects-based monitoring, the plasma proteome offers many unique advantages: it provides a snapshot of whole organism health, sample preparation is easy, and there is potential for non-lethal sampling methods. Recent advances in analytical instrumental technology, annotated sequence databases, and bioinformatic software have made possible the use of high throughput proteomics for environmental monitoring. We have developed a highly cost-effective and efficient method for analyzing the plasma proteome using formic acid protein digestion and liquid chromatography-mass spectrometry. We have used this method to perform proteomic analyses on plasma sampled from multiple teleost fish species in both laboratory and field settings in support of effects-based monitoring and other priority programs at Environment Canada. We have found that experimental design and statistical analyses are key to the successful application of Omic technologies in environmental monitoring. In particular, special care should be taken when considering the number of samples used in a study and the effects of pooling. Particular attention must be given to the selection and definition of what constitutes acceptable or representative reference conditions in the field. Our presentation will describe some of our recent studies from the perspective of what worked, the challenges we encountered, and the lessons we learned from addressing those challenges.

Field-based metabolomics for assessing contaminated surface waters (PL)

Drew Ekman[1], Gerald Ankley[1], Jon Beihoffer[1], Jenna Cavallin[1], John Davis[1], Kathleen Jensen[1], Michael Kahl[1], Kristen Keteles[1], David Skelton[1], Quincy Teng[1], Daniel Villeneuve[1] and Timothy Collette[1]

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Metabolomics is rapidly becoming established as an effective tool for studying the responses of organisms, such as fish, to various chemical contaminants. For example, the literature contains a number of laboratory-based studies involving analysis of samples from organisms exposed to individual chemical toxicants. These lab studies have established the ability of metabolomics to rapidly screen and prioritize individual chemicals for adverse effects, and also to inform toxic modes of action. However, few

examples currently exist of metabolomics being applied for the characterization of biological responses as a result of exposure to complex “real-world” chemical mixtures, or for biomonitoring in the natural environment. This is unfortunate, because metabolomics is well suited for these applications as well. For example, metabolomics can be highly effective in the study of responses to environmental stressors in ecologically-relevant species for whom a fully sequenced and annotated genome may not be available. Also, it can be applied with relatively low per-sample cost, is “open-ended” (requiring no pre-selection of targets), and is highly reproducible with modern nuclear magnetic resonance and mass spectrometry instruments. Finally, biological matrices (e.g., urine) that can be sampled in a minimally-invasive manner are suitable for use in metabolomics studies, opening up the possibility for sampling larger numbers of individuals in wild populations or for repeated sampling of individuals. Recognizing that these are considerable advantages for *in situ* effects-based monitoring, we have been conducting numerous biomonitoring studies with metabolomics in, and around, various water bodies in the U.S. (e.g., Great Lakes). Most of this work involves caged fathead minnows (*Pimephales promelas*), which are strategically deployed at various sites in relation to point and non-point sources of contamination (e.g., wastewater treatment plants, agricultural operations, etc.) Where feasible, the metabolomic data are linked with other omic measurements, targeted bioassays, classical whole-animal outcomes, and site characterization and chemical monitoring data. These studies, which will be discussed here, clearly indicate the substantial potential for effects-based monitoring with metabolomics.

Sediment and Soil Toxicity Method Development and their Application

Utilization of the high water-sediment ratio test in combination with the Metal Effects Addition Model (MEAM) for site-specific assessment and Toxic Identification Evaluation (PL)

Warren Norwood[1]

[1]Environment Canada

In 1999, Borgmann and Norwood outlined a sediment toxicity test method using static, large water-to-sediment ratios (the Cone Test) as an alternative to water renewals in standard sediment tests. In 2013, Norwood et al published a model, the metals effects addition model (MEAM), that can predict the impact of mixtures of ten common metals based on their bioaccumulation. This presentation will demonstrate how the Cone Test in combination with the MEAM can be used to identify (a) metals of concern (increased bioavailability); (b) acute and chronic toxicity; and (c) possible causes of the toxicity and a Toxicity Identification Evaluation (TIE). This will be achieved by describing how to obtain acute (7-day) and chronic (28-day) bioaccumulation and toxicity data from the Cone Test, then how to use the MEAM, now available in a user-friendly Microsoft Excel spreadsheet program. This process can identify which of the ten metals was responsible for any observed toxicity or if other contaminants may be responsible. A number of case studies will be used to demonstrate the process.

Hexagenia spp. as a standardized sediment toxicity test species: How far we have come and where we still need to go (PL)

Trudy Watson-Leung[1]

[1]Ontario Ministry of the Environment and Climate Change

Hyalella azteca and *Chironomus dilutus* are the most commonly used, and currently considered to be the only standardized, sediment toxicity test species. This is because their relative sensitivity is well known, and round-robin testing has confirmed the robustness of their test methodologies. The burrowing mayfly, *Hexagenia* spp., has been regularly incorporated into sediment quality assessments in the province of Ontario, Canada, for almost 25 years to support the sediment toxicity line of evidence in the Canada-Ontario Agreement (COA) Respecting the Great Lakes decision-making framework and the Environment Canada BEAST (BEnthic Assessment of SedimenT) protocol for assessment of Great Lakes contaminated sediment. While the Ontario Ministry of the

Environment and Climate Change methodology for testing with *Hexagenia* spp. has not been updated since 1992, considerable knowledge about the sensitivity, repeatability, and value and limitations of this organism as a toxicity test species has been gained over the years. In this presentation the author will discuss method development and method improvement, advances in culturing techniques and knowledge of species biology, relative species sensitivity, method performance, quality control, and advances toward method standardization.

You are what you eat: The effectiveness of three diets for rearing *Hyalella azteca*, and the influence of diet on organism response to ammonia (PL)

Lisa Kennedy[1] and Trudy Watson-Leung[1]

[1]Ontario Ministry of the Environment and Climate Change

Diets play an important role in determining the overall health of an organism, as this may influence the ability to obtain enough healthy organisms to complete toxicity testing, and possibly alter the organism response to toxicants. It is crucial to have confidence in the health of organisms used, in order to be certain that poor health isn't potentially biasing any results. Three *Hyalella azteca* cultures have been reared on three different diets since birth, reflecting the recommended diets of various investigators. The three diets consisted of: (a) fish flakes (Nutrafin®); (b) a mixture of fish flakes supplemented with the salt water diatom *Thalassisira weissflogii*; and (c) a mixture of fish flakes and yeast, trout chow and cerophyl (YCT). To standardize the amount of food provided to each culture, an equal caloric content of each diet was used. Performance of each diet was evaluated through a 42-day, water-only reproduction, growth and survival test. The three diets will also be evaluated through the organism's response to standard 96-hour toxicity testing with ammonium chloride at three different pH values (8.5, 7.5 and 6.5) as well as at three different temperatures. In the future, toxicity testing will also be conducted with a range of salts and metals to provide further guidance when deciding upon a standardized *Hyalella* diet.

Interlaboratory comparability of gene expression data for Toxicity Identification Evaluation (PL)

Steven Bay[1] and Doris Vidal-Dorsch[1]

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Advances in molecular toxicology hold promise for the development of more sensitive and powerful tools for environmental studies. Yet little is known regarding the reliability of these methods when used for monitoring, especially whether results are comparable among laboratories. We evaluated the interlaboratory comparability of invertebrate gene expression analyses for use in a potential new method for stressor

identification in toxicity tests. A 15K gene expression microarray was developed from the transcriptome of the marine amphipod, *Eohaustorius estuarius*, a species widely used for sediment toxicity assessment. Prior studies using this microarray have identified distinctive patterns of gene expression in *E. estuarius* exposed to different types of sediment toxicants, indicating good potential for use as a molecular TIE method. Six Canadian and U.S. laboratories used the *E. estuariu*s microarray to analyze a common set of tissue and RNA extract samples. The samples represented amphipods exposed to either control or cyfluthrin-spiked sediment. Results showed that probe intensities were similar among laboratories and that data from RNA extracts were highly correlated. Differential gene expression concordance ranged from 0.4 to 0.7 between labs, with the extracts showing greater concordance than tissue samples extracted separately at each lab. Several factors affected data comparability (e.g., instruments and protocols used, personnel expertise). Despite the interlaboratory variability, a subset of genes was consistently differentially expressed across all laboratories. A higher degree of data agreement was observed in differentially expressed genes with a fold change greater than 2. The results indicate that, with control of certain factors, gene expression analyses provide reliable data for assessing contaminant impacts on aquatic organisms.

Taking the bug out of the mud: Water-only toxicity of salts and metals to two size ranges of *Hexagenia* spp. (PL)

Kim Mahon[1], Kathleen Stevack[2] and Ryan Prosser[3]

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Hexagenia spp. is a standard test organism used routinely in sediment toxicity testing. *Hexagenia* are valuable test organisms based on their ecological importance. *Hexagenia* are good candidates to be utilized in water-only test methods; however, little is known about *Hexagenia*'s sensitivities to water-only chemical exposures. In order to investigate sensitivity to chemicals, *Hexagenia* were exposed to a battery of compounds through acute water-only exposures. Toxicity tests were conducted based on the standard Environment Canada acute lethality test method for evaluating the toxicity of effluents to *Daphnia magna* with the following modifications: 96h duration, 23 ± 2°C, and silicone/glass tubing for substrate. *Hexagenia* of two size ranges were used in testing (4-6 mg wet weight (ww) and 20-30 mg ww). Exposures were comprised of a variety of chlorinated and non-chlorinated salts, and metals. Control mortality and the LC_{50} were calculated for each compound. Mean control mortality was less than 5% across all exposures. In three out of the seven salts examined, smaller *Hexagenia* were more sensitive than larger *Hexagenia*. Among all the metals examined, smaller *Hexagenia* were more sensitive than larger *Hexagenia*. The results from this study suggest that *Hexagenia* are not very sensitive to some metals. The LC_{50} for *Hexagenia* exposed to some metals were at least 25% greater than those reported among species of trout. The findings of this study support method development for a water-only *Hexagenia* test, and provide

sensitivity data necessary to strengthen the *Hexagenia* sediment toxicity method and bioaccumulation method.

A paleolimnological investigation of phytoplankton trends in a narrow river-valley reservoir (PL)

Timothy Tse[1], Lorne Doig[1], Zoraida Quinones-Rivera[1], Peter Leavitt[1], Garry Codling[1], Brett Lucas[1], Karsten Liber[1], John Giesy[1], Howard Wheater[1] and Paul Jones[1]

[1]University of Saskatchewan

Paleolimnology can be used to reconstruct long-term environmental trends in reservoirs and lakes. However, only a limited number of cores, often one, are typically collected to characterize a water body. This may be appropriate for morphologically simple lakes, but it may not provide adequate spatial coverage for dammed river valley reservoirs which often have distinct physical, chemical and biological gradients. Using Lake Diefenbaker, a 225-km long reservoir in southern Saskatchewan, Canada, as a test case, this study reconstructed long-term trends in primary productivity within the context of these spatial gradients. Cores of sediment were collected from the main channel along the longitudinal axis of the reservoir and sectioned vertically into 1-cm increments. Fossil pigments, representative of a diversity of taxonomic groups, were extracted, identified and quantified. Trends in pigment concentrations in recent surface sediments (0-1 cm) suggest an increase in algal productivity with distance down-reservoir, with greatest primary production in the mid-reservoir region. Concentrations of pigments in the vertical profiles of reservoir sediments suggest increasing primary productivity over time only in down-reservoir areas. An increasing trend of the pigment myxoxanthophyll, produced by filamentous and colonial cyanobacteria in the areas furthest down-reservoir, suggests increasing production of potentially harmful cyanobacteria. Overall, these results demonstrate the usefulness of paleolimnology in reconstructing long-term trends in reservoirs and the importance of investigating both spatial and temporal variability when assessing algal abundance or productivity of a dammed river-valley reservoir.

Test suite to determine soil microbial health (PL)

Stephanie Kvas[1], Jessica Rahn[1], Lee Beaudette[1] and Rick Scroggins[1]

[1]Environment Canada

The microbial community in soil is important on many levels, including organic matter decomposition, nutrient cycling, and the health of the ecosystem. Therefore, it is important to consider how the microbial community is affected when contaminants are present in soil. Despite the importance of the microbial community, ecological risk assessments do not typically include soil microbial health. The Soil Biotechnology

Laboratory (SBL) at Environment Canada is part of the Biological Assessment and Standardization Section, whose goal is to develop and standardize biological test methods. The SBL recently developed a comprehensive test suite in conjunction with a data integration method for assessing soil microbial health. The test suite encompasses experiments that assess biomass, activity and diversity of the soil microbial community. Following these experiments, the data integration method is used to conclude whether the microbial community has been un-impacted, impacted, or severely impacted by the contaminant or contaminant mixture. Heterotrophic plate count and fumigation-extraction methods are used to assess microbial biomass. Organic matter decomposition, nitrification, respiration, and bait lamina tests are used to assess microbial activity in soil. Enzyme assays, community-level physiological profiling, and denaturing gradient gel electrophoresis are used to assess diversity and community structure in soil. The long-term goal of our research is to standardize this test suite and data integration method so that soil microbial health may be reliably and consistently assessed in any soil type. Here, we present our most recent results, using our test suite, on the soil microbial health of three soils: a petroleum hydrocarbon (PHC)-contaminated boreal forest soil, a PHC/metal-contaminated soil, and a metal-contaminated soil.

Laboratory vs. field-based toxicity of nickel to soil organisms: A critical review (PL)

Beverley Hale[1], Yamini Gopalapillai[1], Chris Schlakat[2], Mike McLaughlin[3], Julie Kikkert[1], Wilson Lau[1] and Tyson Jennett[1]

[1]University of Guelph, [2]Nickel Producers Environmental Research Association, [3]Commonwealth Scientific and Industrial Research Organisation

Field studies at nickel (Ni) refining and smelting operations in Port Colborne, Ontario, and Sudbury, Ontario, as well as the "Metals in Asia" (MIA) study, have suggested that bioavailability and toxicity of aged Ni in field soils is lower than in soils amended with soluble Ni species, even after leaching and aging. The European Union risk assessment (EU RA), which determined a bioavailability-based HC_5 (50%) for soil organisms exposed to Ni-amended soils, included an Application Factor (AF) of 2 because there was no validation by higher-tier data. The toxicity thresholds determined in these field studies were not greater than, and in most cases much less than, those identified in the EU RA. Mineralogical studies of soils amended with soluble Ni forms have identified that even with aging, the Ni species in smelter- or refinery-contaminated soils (i.e., spinels) are less bioavailable. This is confirmed by the very large difference in toxicity thresholds for plant growth in anthropogenically-contaminated soils when expressed as extractable-soil [Ni] compared to total-soil [Ni], a gap that is much smaller in soluble Ni-amended soils. The conclusion of the review is that the outcome of the EU RA is a conservative screen, suitable for prospective RA. However, for retrospective, site-specific RA, the bioavailability-based HC_5 may be exceeded, substantially, without there being a true risk, depending on the original source of the Ni and the current speciation. Such

information, as well as field-based ecotoxicity data, needs to be collected for site-specific RA and then thoughtfully integrated into the decisions surrounding risk management; otherwise there is a risk of costly, unnecessary mitigation.

Site-specific management objectives for petroleum hydrocarbons in urban soils of Arctic Canada (PL)

Steven Siciliano[1] and Phillip Garvey[1]

[1]University of Saskatchewan

Developing site-specific management objectives for Canadian soil ecosystems can substantially reduce the cost and increase the sustainability of remediation and management plans. However, for many urban sites, soil collection protocols are not well suited to existing soil testing protocols and further, there is little published data on the suitability of the proposed Tier 2/3 protocols for site-specific management objective development. Here we investigated a site in Iqaluit, Nunavut, with petroleum hydrocarbon (PHC) concentration in the soil exceeding Tier 1 soil quality guidelines. Our goal was to adapt the Draft Tier II ecological risk assessment guidance developed during the 5-year review of the CCME PHC protocols in 2006 for Nunavut and urban soil investigations. The toxicity of PHC was assessed using a series of toxicity assays to develop dose-response curves for the contaminants present at the site. Site soils containing a gradient of PHC concentration were assessed initially using nitrification and collembola toxicity assays. Following this, site soils were diluted to create a full toxicity range and assessed using oribatid mites, collembola, enchytraeids, and nematodes, all of which are ubiquitous in Canadian soils, as well as the soil enzymes dehydrogenase, arylsulfatase, and acid phosphatase. In addition, Environment Canada plant protocols were modified to cope with the limitations of soil sampling in Arctic and urban regions. The Draft Tier II protocol with its Phase 1 and 2 steps was very effective in assessing site risk within a 3-month period. Further, our modifications to existing test methods allows one to use soil volumes commonly collected during site investigations. Thus, our work has developed a protocol that will allow site managers to use soil samples, typically collected for site characterization, to also provide site-specific toxicity results.

Bridging the gap between research and risk assessment in terrestrial ecosystems (PL)

Juliska Princz[1], Ellyn Ritchie[1], Jessica Velicogna[1], Christopher Fraser[1] and Rick Scroggins[1]

[1]Environment Canada

Canada has committed to the assessment of environmental and human health risks for priority chemical substances as part of Canada's Chemicals Management Plan

(CMP). The assessments are usually based on available experimental data and predictive modelling tools, with focused research conducted to fill data gaps where feasible. Gaps exist for the soil compartment, particularly for chemical substances known to partition to soil, for which the persistence, bioaccumulation and inherent eco-toxicity potential are uncertain. As a result, research efforts have been focused on filling some of these information gaps for various organic and inorganic chemical substances (e.g., siloxanes, chemical dyes, metals). Case studies will be presented to demonstrate how standard soil test methodologies are used to provide effect estimates and confirm model predictions for the risk assessment and management of chemical substances in the environment. For example, the importance of bridging the gap between mechanistic laboratory data and model predictions for the profiling of ionic organic substances will be presented, as in some instances, even limited bioavailability combined with reactivity can result in exposures not predicted by traditional modelling methods. Another example will focus on the use of metal moiety-based evaluations whereby a common constituent across a group of substances is used for risk assessment purposes, with the assumption that the constituent has the greatest toxicological significance. As a result, the evaluation of a group of priority substances sharing a common metal moiety, zinc (Zn), will be discussed. The results of these studies demonstrate the importance of research for the development and confirmation of tools applicable to better understanding and predicting the impacts of chemicals in the environment.

Review of technical issues and test performance of Environment Canada's test for toxicity of contaminated soil to earthworms (PL)

Jennifer Miller[1], Gladys Stephenson[2], Kelly Olaveson[2], Emma Shrive[2], Juliska Princz[3], Jessica Velicogna[3], Ellyn Ritchie[3], Muriel Jatar[3], Chris Fraser[3], Leana Van der Vliet[3] and Rick Scroggins[3]

[1]Miller Environmental Sciences Inc., [2]Stantec, [3]Environment Canada

Since its publication in 2004, Environment Canada's (EC's) earthworm test method, EPS 1/RM/43, has been used by a number of government and private sector testing laboratories for soil toxicity testing. After 10 years of application, however, EC's Biological Assessment and Standardization Section has received feedback on the conductance of this method and concerns have been raised regarding the relatively high level of test validity failures and data variability in the test for effects of prolonged exposure on survival, reproduction, and growth. A review of the past test method performance was conducted using data from two laboratories to investigate various technical issues and overall test performance. The three main study objectives were to: (a) determine the frequency of invalid tests and the possible source(s) of variability, primarily in control (natural and artificial) and reference soils; (b) determine the relative sensitivities of the reproduction and juvenile growth endpoints; and (c) determine the appropriate test power for the earthworm reproduction growth test. In addition, standard operating procedures were examined and anecdotal information was collected

from technical staff from the two laboratories to assess possible sources of variability associated with the methodology. The results of this data review, possible sources of test performance variability, and endpoint sensitivity differences will be discussed.

Development of a plant toxicity test using species native to the Canadian boreal wetlands (PL)

Mary Moody[1] and Rick Scroggins[2]

[1]Saskatchewan Research Council, [2]Environment Canada

Protection of vulnerable wetlands across Western Canada is an important aspect of resource development. Development of a plant toxicity test specific to wetland habitats of the boreal eco-zone is a priority recognized by governments and industry in Canada. Hundreds of accidental spills of crude and produced water from oil and gas production pipelines occur in wetlands across Western Canada each year. Therefore there is a need for relevant testing tools to quantify biological impacts from pipeline spills or poor waste treatment practices which lead to releases of high levels of salts and hydrocarbons into wetland environments. Candidate test species native to three wetland habitat types (bogs, fens and shallow water marshes) were chosen to offer an ecologically relevant test battery. Case studies of three impacted wetland sites are used to illustrate the relative sensitivity of the test species using measurements of growth in dilutions of substrates and waters. The goal of the research and validation efforts is development of a plant testing method using ecologically relevant species that are capable of quantifying the impacts on vulnerable wetland habitats.

Development of field deployable assays to monitor and quantify the effects of waterborne contaminants on wetland plants and associated soil fungi (PL)

Kevin Stevens[1], Kevin Maccoll[1], Bishnu Twanabasu[2] and Barney Venables[2]

[1]Wilfrid Laurier University, [2]University of North Texas

Water quality impairments impinge on valued wetland ecosystem functions through their impacts on wetland vegetation. Quantifying and monitoring plant responses to waterborne contaminants are necessary to maintain, enhance and restore wetland ecosystem function. Existing approaches utilizing greenhouse/growth room bioassays do not capture temporal heterogeneity in water quality, while soil/sediment/water chemistry analyses do not reflect bioavailability of a given compound. We are developing a suite of field-based assays to monitor and assess the effects of waterborne contaminants on wetland plants and associated soil fungi. Our assays include established endpoints (seed germination and biomass) and less frequently used endpoints (root morphology, arbuscular mycorrhizal spore germination and hyphal growth). Because our assays are conducted on-site we are able to obtain a direct assessment of soil/water

toxicity using species of known providence and behaviour. Furthermore, our assays permit the assessment of a range of wetland plant species from emergent to submerged macrophytes. Our results are site-specific, reflect bioavailability, and circumvent many of the known drawbacks associated with existing field- and laboratory-based assessments.

Relationships among comparative methods for measuring the bioavailability of weathered petroleum hydrocarbons to ecological receptors in contaminated soil (PL)

Robin Angell[1], Gladys Stephenson[2] and Barry Zajdlik[3]

[1]Monsanto Canada Inc., [2]Stantec, [3]Zajdlik & Associates

The toxicity of petroleum hydrocarbons (PHCs) to ecological receptors in soil can be dramatically different between fresh and aged/weathered products. The processes of aging and weathering can result in the sequestration of PHCs, resulting in relatively recalcitrant PHC residuals in soil. The risk associated with exposure of ecological receptors to these recalcitrant PHC residuals may be more accurately estimated when PHC bioavailability is taken into consideration. Two extraction methods, hydroxypropyl-β-cyclodextrin (cyclodextrin) and the SEG (Simulated Earthworm Gut) assay, were used to measure the bioavailability of PHC Fraction 2 (>nC10 to C16) to ecological receptors. Artificial soils were spiked with distilled F2 and weathered for 168 days. The soils were extracted with both cyclodextrin and the enzyme mixture (SEG assay). Toxicity tests using plants and invertebrates were also conducted with the weathered F2-spiked soils. Cyclodextrin-extractable and SEG-extractable PHC concentrations were compared to the PHC concentrations measured in soil using solvent extraction and GC-FID (gas chromatography coupled with flame ionization detection) following CCME methods. The relationships between the biological responses of a battery of single-species toxicity tests with these same soils and the bioavailable and total petroleum hydrocarbon concentrations were examined and compared. The results of the comparisons indicated that all extraction methods were correlated with the results of the toxicity tests. The relative value of these extraction methods will be discussed.

Comparison of test results for agronomic and boreal forest/wetland species used as separate lines of evidence in Alberta Tier 2 risk assessments (PL)

Gladys Stephenson[1], Kathryn Bessie[2], Mary Moody[3] and Robin Angell[4]

[1]Aquaterra Environmental Consulting Ltd., [2]Tetra Tech EBA, [3]Saskatchewan Research Council, [4]Stantec

Environment Canada (EC) has been developing ecotoxicity test procedures for upland boreal forest plant, invertebrate, and microbial organisms, as well as for wetland plants. EC published the methods for boreal forest species in August 2013 (e.g.,

"Biological Test method: Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Forest"). Test results for these species and test procedures can be used to support site-specific (Alberta Tier 2 and CCME Tiers 2 and 3) risk assessments of contaminated lands. Ecotoxicity assessments of contaminated soil were contracted by Tetra Tech. For these assessments, the Saskatchewan Research Council (SRC) used upland boreal forest plant species that included white spruce (*Picea glauca*), aspen poplar (*Populus tremuloides*) and bluejoint reedgrass (*Calamagrostis canadensis*), and Stantec used agronomic plant species that included alfalfa (*Medicago sativa*), northern wheatgrass (*Elymus lanceolatus*), and barley (*Hordeum vulgare*). Upland Dark Gray Luvisolic soils (Bearberry and Hubalta soil series) and wetland Terric Mesisols or Terric Humisols in Alberta (AB) were contaminated with petroleum hydrocarbons from landfarming (biotreatment) of oilfield wastes. These soils have residual weathered aged hydrocarbons, some metals, and a trace of salts. Biotreatment additives included sawdust, bark chips and fertilizer. The results of the ecotoxicity testing with boreal forest species were compared to those for agronomic species by comparing the result of using these data to determine if this site meets the AB Tier 2 Pass/Fail Criteria (Draft 2007) or, alternatively, the Canadian Council of Ministers of the Environment's risk assessment goal that cumulative negative effects be less than 25%. The challenge of separating non-contaminant effects (e.g., those attributable to the influence of physico-chemical variables) from contaminant effects (e.g., metals or PHCs) will be discussed in addition to the importance of the reference negative control soils. The implications of the use of site-specific toxicological data for adaptive reclamation will be presented.

Alternative Tier II site-specific remedial objectives (SSROs) that acknowledge non-contaminant effects (PL)

Barry Zajdlik[1] **and Gladys Stephenson**[2]

[1]Zajdlik & Associates Inc., [2]Stantec

In Canada, Tier II soil petroleum hydrocarbon-contaminated (PHC) guidelines allow for limited modifications to account for site-specific variables. However the most sensitive pathway that invariably drives PHC guidelines is direct soil contact and for this pathway, modification by site-specific variables is disallowed. We analyzed soil toxicity test data from three PHC-contaminated sites using a combination of data reduction and model averaging procedures collectively described as data reduction and model averaging (DRAMA) in order to objectively deal with multicollinearity and model selection issues. DRAMA results showed that non-contaminants were more strongly correlated with observed toxicity tests responses than PHCs and even the effect of a specific site. The DRAMA procedure may be useful in deriving generic equations describing how pedologic variables affect PHC toxicity. This can allow for derivation of site-specific remediation objectives for total PHCs.

Risk-based management of site soils contaminated with a mixture of hazardous substances: New methodological approach and case study / Gestion basée sur les risques de terrains contaminés par des mélanges de substances toxiques: Nouvelle approche méthodologique et étude de cas (PL)

Agnès Renoux[1], Barry Zajdlik[2] and Gladys Stephenson[3]

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A novel approach to reduce the uncertainty associated with the assessment of ecotoxicological risks and to allow the determination of remedial objectives was developed for a scenario involving multiple contaminants in soil. This new approach used laboratory-derived, site-specific, toxicological data (i.e., obtained from toxicity testing using species in direct contact with soil such as plants and invertebrates) instead of the more traditional generic toxicological benchmarks for corresponding groups of organisms. This approach took advantages of statistical models (data exploration and reduction; generalized linear models) to derive tools (equations) that could predict the level of impairment associated with any combination of metal concentrations measured on site (without ignoring confounding factors) and compare it to a pre-specified acceptable threshold. A case study will be presented whereby this method was applied to a large site contaminated with a mixture of metals. Ultimately, the distribution of predicted levels of risk for both soil invertebrates and plants was determined for the entire site and compared to those obtained with the traditional approach using benchmarks.

Une nouvelle approche visant à réduire l'incertitude associée aux évaluations de risques écotoxicologiques et à permettre la détermination d'objectifs de réhabilitation a été développée pour des cas de contamination multiple du sol. Cette nouvelle approche utilise des données toxicologiques spécifiques au site obtenues en laboratoire (c'est-à-dire des résultats de biotests utilisant des espèces en contact direct avec le sol du site tels que des plantes et des invertébrés) plutôt que l'approche plus traditionnelle d'évaluation des risques utilisant des valeurs de référence toxicologiques génériques à des groupes d'organismes. Cette approche profite de modèles statistiques (exploration et réduction des données, modèles linéaires généralisés) pour dériver des outils (équations) permettant de prédire un niveau d'effets associé avec chaque combinaison de concentrations en métaux mesurés sur le site (sans ignorer les facteurs confondants) et de comparer ce niveau à un seuil acceptable prédéfini. Cette méthode a été appliquée à un cas concret de grand site contaminé avec un mélange de métaux. Dans cette étude de cas, la distribution des niveaux de risques prédits pour les invertébrés du sol et les plantes a été déterminée sur tout le site et comparée avec ceux obtenus grâce à l'évaluation traditionnelle des risques utilisant les valeurs de référence toxicologiques.

Evaluating the effects of triclosan on the rhizosphere of field crops in biosolid-amended soil (PL)

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Triclosan (TCS) is a persistent antimicrobial agent commonly found in personal care products that include soaps, shampoos, and other sanitation goods. Due to its widespread consumer use and dermal application, relatively high concentrations are found in municipal wastewater and wastewater sludge (biosolids) ranging as high as 0.133 $mg \cdot g^{-1}$. Specifically, TCS interferes with bacterial uptake of nutrients in plants and can promote microbial resistance in field crops. Recent studies have found a mycotoxic relationship between TCS and arbuscular mycorrhizal fungi (AMF) colonization in the rhizosphere of field crops. This suggests that TCS found in biosolid-amended soil may pose risks to the rhizosphere of field crops where essential nutrients are exchanged. The purpose of this study is to characterize the effects of TCS on the growth of field crops, as well as the symbiotic colonization of their roots by AMF in soils amended with different types of biosolids. Data from initial 90-day tests with soybean suggest no significant TCS concentration-dependent effects on crop emergence, shoot length, or biomass. Analyses of root structure and AMF colonization, as well as bioaccumulation, have yet to be completed. Testing with other crop species (corn and spring wheat) is ongoing, and will help to better characterize whether biosolids land application poses a risk to crop and human health.

Multi-source stressors on Great Lakes ecosystems (PO)

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Multiple stressors within the Great Lakes can lead to cumulative effects throughout various ecosystems. In this study, five chemically contaminated sites within the Great Lakes and associated watersheds are being investigated to quantify the toxicity and bioavailability of legacy contaminants such as dichlorodiphenyltrichloroethane (DDT), dioxins, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), as well as emerging contaminants such as personal care products and pharmaceuticals. The three lake sites include Great Lakes Areas of Concern (AOC). These lake and watershed sites represent contamination within Ontario originating from different interacting chemical sources including municipalities, large/small industries, and agriculture, each characterized by a unique suite of chemicals of concern. The fate and effects of contaminants at these sites are being categorized using a sediment quality quadrad approach utilizing information on bulk chemistry, benthic invertebrate community structure, sediment toxicity and bioaccumulation. In addition, forage and sport fish tissue residues and aquatic habitat assessments will be used to augment the evaluation of each

site and to inform monitoring and remediation programs. This project is in part being conducted as a component of ongoing comparisons of temporal toxicity changes to previous evaluations. The data will be used to generate separate risk assessments for each site, aiding in management decisions regarding Great Lakes protection and contaminated site remediation.

Comparison of three methods for extracting sediment porewater (PO)

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Many factors influence the response of aquatic organisms to toxicants. One factor is the location of an organism within an aquatic environment. Organisms that occupy both the sediment and the sediment/water interface are popular test organisms for assessing contamination of sediment. These organisms can be directly associated with porewater or interstitial water, water that lies in between the spaces of particles in sediment. This water is in constant contact with the sediment and the overlying water. Interstitial water also has the ability to partition out of the sediment into the aquatic system and reach equilibrium with the surface water or ground water. There currently is little guidance on how to collect/extract porewater from sediment samples. This study examined how different approaches of collecting porewater influence measured pH, ammonia, and contaminants of concern. Three different extraction protocols were conducted on three different sediments, stored for three different periods of time. The extraction techniques included centrifugation, removal of settled water, and removal of settled water followed by centrifugation. The results indicated no difference among pH and ammonia results across all treatments, while there was high variability among measured metal and polycyclic aromatic hyrdocarbons (PAH) levels. Most notably there were elevated levels of PAH compounds in both of the centrifuged treatments compared to the settled water. Results suggest that among the three different extraction protocols examined, there is little impact on pH and ammonia measurements. However, if it is of interest to examine contaminants of concern in the porewater, the extraction method should be taken into consideration.

A comparison of the toxicity of zinc moieties to terrestrial plants and invertebrates in a sandy soil (PO)

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Inorganic moieties of concern identified as a priority under the Government of Canada's Chemicals Management Plan (CMP) II were evaluated by Environment Canada's

Biological Assessment and Standardization Section (BASS). As there was a large volume of chemicals requiring evaluation under CMP initiatives, a metal moiety-based approach was recommended to streamline the risk assessment process, with Zn-containing substances selected as the first metal-moiety for soil assessment. To support a moiety-based initiative, an evaluation of the toxicological effects of physical and chemical groups was assessed through the use of representative substances: (a) an organometal (zinc diethyldithiocarbamate (ZDDC) (CAS 14324-55-1)); (b) a water-soluble inorganic salt (zinc chloride ($ZnCl_2$) (CAS 7646-85-7)); (c) a water-insoluble inorganic (zinc oxide (ZnO) (CAS 1314-13-2)); (d) a water-soluble organic metal salt (benzenediazonium, 4-chloro-2-nitro-, tetrachlorozincate(2-) (BCNZ) (CAS 14263-89-9)); (e) a water-insoluble organic metal salt (zinc stearate (ZnSt) (CAS 557-05-1); and (f) the organic moiety of zinc stearate (stearic acid (SA) (CAS 57-11-4). All of the substances were assessed using definitive plant (*Elymus lanceolatus* and *Trifolium pratense*) and invertebrate (*Folsomia candida* and *Eisenia andrei*) tests in a sandy soil. Soil samples were analyzed at the beginning of each test so that effective concentrations could be derived on a nominal basis, a total Zn basis, and on a total parent compound basis for select chemicals (e.g., ZnSt, BCNZ and ZDDC).

Probability distributions of *in vitro* bioaccessibilities of nickel from multiple studies: A risk assessment tool that is less than site-specific but more than generic (PO)

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Health Canada (2010), in their guidance for proponents wishing to conduct a detailed quantitative risk assessment (DQRA) suggest that bioaccessibility/bioavailability be determined for a range of soil particle sizes; bioaccessibility/bioavailability be determined for several volume/mass ratios of simulated gastric fluid and soil; and bioavailability of the element in the study that formed the basis of the reference dose (RfD) or reference concentration (RfC) be determined. Data on bioaccessibility of Ni in a range of soils and particle sizes could be aggregated into probabilistic estimates of bioaccessibility that could be used in screening-level risk assessments instead of the single-value default or site-specific values. Using a number of soils with elevated concentrations of aged Ni, this study explored whether relationships were robust if particles from a variety of sources were included. It also asked the following questions: is the bioaccessibility of the high total [Ni] particles suppressed by insufficient acid/soil ratios during digestion? Or are there actually two separate populations of "bioaccessibility" that can be distinguished by Ni mineralogy or speciation? How would this log-normal relationship between total [Ni] in particles and its bioaccessibility be reflected in a correlation between bioaccessibility and bioavailability? The data suggest that the bioaccessibility and bioavailability of Ni varies according to speciation, which affects solubility. In mineral soils spanning a wide range of concentrations and speciation, a common leaching curve exists, but is complex. This relationship offers an approach to

incorporate bioaccessibility into risk assessment of contaminated soils at a screening level that is technically more robust than current approaches.

Unconventional Oil and Gas

U.S. experience with assessing and regulating potential impacts from the development of unconventional oil and gas resources (PL)

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With recent advances in hydraulic fracturing (fracking) and horizontal drilling, the onshore oil and gas industry in the U.S. has experienced significant change. The upshot: extraction of oil and gas from unconventional resources is more technologically and economically feasible than before, and many are predicting that the U.S. and Canada combined will be self-sufficient in natural gas, gas condensates, and crude oil before 2020. The rapid development of unconventional resources has raised concerns with regards to potential impacts on public health and the environment. From an aquatics perspective, there are concerns over four main exposure pathways that could cause drinking water pollution: (a) fracking chemicals could enter drinking water aquifers directly due to improper well construction or an over-aggressive "frack"; (b) the potential for pollution from the vast amounts of produced water and flowback, which the industry sends to publicly-owned treatment works (POTW), discharges into surface waters, or injects back into the ground; (c) surface drilling operations create the potential for spills and leaching of harmful waste products into groundwater; and (d) the fracturing of underground rock formations could potentially cause oil and gas reservoirs to communicate with groundwater aquifers. The first three exposure pathways are particularly important because the chemicals used in the hydraulic fracturing process may include chemicals known to be toxic to humans and wildlife, and known carcinogens.

These potential risks have resulted in a number of amended and proposed environmental regulations and guides at the federal, state and local levels. Examples related to water quality include the U.S. Environmental Protection Agency (EPA)'s proposing fracking fluid disclosure rules, discharge standards for wastewater from unconventional oil and gas extraction, and updated water quality criteria for chloride. The EPA is also undertaking a national study to understand the potential impacts of hydraulic fracturing on drinking water resources, with a final draft report expected to be delayed until 2016. Public concern over the potential risks has also spawned numerous efforts to enact drilling moratoriums and even bans on fracking at the state and local levels. This presentation will provide an overview of the technologies involved in the U.S. in accessing unconventional oil and gas resources and the results of assessments of such technologies with respect to aquatic resources. It will provide some commentary on issues in play when determining whether the risks are "known" or "potential" based on U.S. literature accounts. Finally, the presentation will provide a listing of key U.S. regulatory

developments related to aquatic resources that have been adopted or proposed in response to the unconventional oil and gas revolution.

Impacts of petrochemical activity on the historical deposition of polycyclic aromatic hydrocarbons to lake sediments in northwestern Canada (PL)

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With an ever-expanding global population, the demand for fossil fuels remains strong and development of petrochemical resources continues. Yet very little is known of the impact that the various methods of exploring and exploiting these resources may be having on the deposition of polycyclic aromatic hydrocarbons (PAH) to nearby aquatic systems. PAHs can be highly carcinogenic to humans, and research has shown that the body burden of PAHs in some arctic species has increased since the early 1980s. The PAH profiles (including both substituted and unsubstituted PAHs) in sediment cores from a set of lakes strategically selected for the type of petrochemical they may be impacted by, were examined against the timing of the respective petrochemical developments to determine whether the temporal patterns of PAH deposition in the sediments agree with historical emission patterns. The effects from four different types of petrochemical developments were explored in this study: (a) *in situ* extraction and separation of oil sands (Cold Lake, AB); (b) open-pit oil sands extraction (Fort McMurray, Alberta); (c) abandoned conventional natural gas exploration (Mackenzie Delta Uplands, NWT); and (d) conventional gas and oil extraction (Cameron Hills, NWT). In addition, PAH sources were characterized as either potentially pyrogenic or petrogenic in origin over a time period that extends to pre-industrial times using diagnostic ratios of specific groups of PAHs that can be related to potential sources.

In all study lakes the average concentration of the sum of unsubstituted (parent) PAHs was less than the sum of substituted (alkyl) PAHs, except for the *in situ* extraction sites where the reverse was true, suggesting that the *in situ* sites are dominated by pyrogenic sources of PAHs. With the exception of sediments near open-pit mining, all sediment cores show no clear trend in PAH deposition that corresponds to the timing of their respective petrochemical developments. The open-pit oil sands sites show increasing concentrations of PAHs that coincide with the timing of the onset of open-pit oil sands mining. Indeed, the open-pit cores demonstrate a clear shift from pyrogenic sources of PAHs (primarily wood and coal burning) in sediments from about the 1950s to petrogenic sources in more modern sediments. These trends were also seen when surface sediments samples from all sites were compared in a principal components analysis (PCA); the different sites were clearly grouped by region, with conventional sites clustering around parent compounds, suggesting pyrogenic sources, and the open-pit sites clustering around alkylated compounds, suggesting petrogenic sources. A deeper

understanding of the effects of different types of petrochemical developments on PAH deposition to lake sediments will allow us to build a clear picture of how these contaminants may be entering and cycling through the environment, the food web and, ultimately, us.

Mixtures of metals and polycyclic aromatic hydrocarbons: Using *Hyalella azteca* and isoboles to explore ecological risk (PL)

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Mixtures of metals and polycyclic aromatic hydrocarbons (PAHs) occur ubiquitously in aquatic environments, yet relatively little is known regarding their potential to produce non-additive toxicity (i.e., antagonism or potentiation) and additive toxicity. We conducted 48-hour water-only mortality experiments to determine the additive relationships between binary mixtures of metals (i.e., copper, cadmium, vanadium, and nickel) with the PAHs phenanthrene (PHE) and phenanthrenequinone (PHQ) using the aquatic amphipod *Hyalella azteca*. We used a three-dimensional isobole model-based approach to compare the observed co-toxicity in juvenile amphipods to the expected additive outcomes based on concentration addition (CA) and effects addition (EA) mixtures theories at the 50% and 10% effect levels. We found that the additive nature of mortality varied substantially based on which metal and PAH were mixed. A clear case of more-than-additive mortality was observed for copper-PHE, copper-PHQ, cadmium-PHE, and cadmium-PHQ mixtures. Vanadium-PHE, vanadium-PHQ, and nickel-PHE mixtures produced strictly additive-toxicity, and nickel-PHQ mixtures produce less-than-additive toxicity. A comparison of the LC_{10} concentrations for more-than-additive mixtures with Canada's federal water quality guidelines revealed that, regardless of this enhanced toxicity, the guidelines concentrations were still protective for acute exposures.

Toxicity of vanadium to aquatic organisms representative of the Athabasca oil sands region (PL)

Stephanie Schiffer[1], Lorne Doig[1] and Karsten Liber[1]

[1]University of Saskatchewan

Petroleum coke, generated as a by-product during bitumen upgrading processes in the Athabasca Oil Sands (Alberta, Canada) and stored on site, contains elevated concentrations of the metal vanadium (V). Currently, oil sands industries face the challenge of storage and remediation of this ever-accumulating material. Previous studies have demonstrated that toxicologically relevant levels of V leach from coke immersed in water, thus potentially exposing surrounding freshwater systems to elevated concentrations of this metal. However, our current understanding of V toxicity to

freshwater organisms is quite limited. Therefore, this research aims to assess the toxicity of V to various freshwater organisms and to compare the sensitivity of commonly used laboratory test species to species more representative of northern Alberta. Toxicity tests were completed using various freshwater algae, aquatic invertebrates and fish species. Acute and chronic toxicity endpoints for V included lethality, growth, inhibition of reproduction, and emergence of insects. Our results were combined with data from peer-reviewed literature to construct acute and chronic species sensitivity distributions for this metal. To date, acute toxicity (LC_{50}s) for the species tested ranged from 0.60-50.1 mg $V \cdot L^{-1}$ for *Ceriodaphnia quadrangula* and *Chironomus dilutus*, respectively. Sensitive chronic toxicity values (EC_{50}s) included 0.15, 3.7 and 33.9 mg $V \cdot L^{-1}$ for *Daphnia dentifera* reproduction, *Pseudokirchneriella subcapitata* growth, and *C. dilutus* emergence, respectively. Toxicity tests are currently being completed with fish species. Species sensitivity distributions based on V toxicity thresholds suggest that the Cladocera are the most sensitive organism tested to date, and that similar sensitivities have been observed between our standard and comparable field-relevant species. This research provides the data needed for future development of appropriate site-specific water quality guidelines (short- and long-term) for V in the Alberta oil sands region.

Comparison of the toxicity of two commercial naphthenic acids extracts and one oil sands process affected water extract to tadpoles of the West African frog, *Silurana tropicalis* (PL)

Juan Gutierrez Villagomez[1], Kerry Peru[2], John Headley[2] and Vance Trudeau[1]

[1]University of Ottawa, [2]Environment Canada

Bitumen and oil sands process affected water (OSPW) are highly variable in composition, making them difficult to characterize. Naphthenic acids (NAs) are considered the major toxic component of the OSPW and have been detected in the Athabasca River in Alberta. The toxicity and effects of NAs on physical development are still unclear. The aim of this study was to compare the effects of two commercial NA (cNA) preparations and one OSPW extract, on early embryonic development in the West African frog, *Silurana tropicalis*. The data obtained during 3-day exposures showed that the survival was decreased by 60% at 6 $mg \cdot L^{-1}$, but this effect was not observed with the second cNA extract or with the OSPW extract. Tadpole length was decreased by both cNAs. In treatment with 6 $mg \cdot L^{-1}$ cNA, nearly 100% of the tadpoles suffered at least one type of abnormality; however, with the OSPW extract approximately 25% of the tadpoles suffered this outcome. In the 24 $mg \cdot L^{-1}$ OSPW extract treatment, more than 95% of the tadpoles had at least one abnormality. Abdominal edema was the most common abnormality observed, followed by blistering, gut abnormalities, flattened face and oval shaped eyes. The OSPW extract had lower toxicity than the two cNA, suggesting that at least some cNAs are not suitable surrogates for oil sands-derived NA extracts. Research on the relationship between chemical composition, survival, development and growth

in *S. tropicalis* is ongoing. Supported by CONACYT (Mexico) and University of Ottawa Research Chair Program.

Toxicity of the Access Western Blend diluted bitumen to medaka (PL)

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The growth in production and transport of diluted bitumen (dilbit) from Alberta's oil sands has raised concerns about the potential impacts of spills on aquatic ecosystems. However, there are no published data on the toxicity of dilbit to fish. Crude and refined oils have been tested extensively, and impairment of fish recruitment has been associated with toxicity to embryos, a life stage that cannot avoid exposure to spilled oil but is uniquely sensitive by virtue of embryogenesis. As a preliminary assessment, we measured the toxicity of Access Western Blend (AWB) Dilbit to embryos of Japanese medaka (*Oryzias latipes*), a model test species, using two different exposure regimes (i.e., mechanical dispersion (water accommodated fraction; WAF), and chemical dispersion (chemically enhanced water accommodated fraction; CEWAF)). Exposure to the tested AWB concentrations did not increase mortality of medaka embryos, but significantly increased the proportion of malformed animals. Under the tested conditions, Corexit 9500 was effective for dispersing AWB dilbit and increasing the exposure of medaka embryos to the toxic constituents of dilbit. Mechanically dispersed oil (i.e., WAF) was much less toxic than chemically-dispersed oil (i.e., CEWAF). A complementary study is ongoing to identify the molecular mechanism of action by which AWB exerts its effects. Among others, cyp1a mRNA levels increased in a dose-response manner in both WAF and CEWAF exposures, with significantly higher level observed in the highest CEWAF treatment (320-fold change increase).

Larval fish responses to environmental samples from oil sands areas (PL)

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[1]Environment Canada

As part of the Joint Canada-Alberta Oil Sands Monitoring Plan, the toxicology of natural and oil sands-related environmental samples was studied. One of the goals of the toxicity tests is to examine pathways and sources of contaminants that may be causing effects in wild fish and invertebrates. Samples were collected from sites where wild fish health and invertebrate communities were assessed. In this way, linkages could be made between wild invertebrates and fish, in comparison to controlled studies of lab fish

exposed to certain components of the environment (sediment, water, and snowmelt). Embryo-larval fathead minnows (*Pimephales promelas*) were used to assess the chronic toxicity of the following environmental samples: river sediments, river waters, snow melt waters, spring freshet waters, and suspended sediments collected in the vicinity of the Canadian oil sands. Samples were collected in 2009-2014 from rivers near oil sands processing facilities along the Athabasca River and tributaries in areas of oil sands development, and compared to samples collected far from sites of oil sands mining and processing. Fertilized fathead minnow eggs were exposed for 21 days (through hatch to 7-15 days post-hatch) to samples in dose-response gradients. Most environmental samples caused no effects in larval fish in 21-day assays. Samples that caused effects in larval fathead minnows were several snow melt samples, sediments from the Steepbank and Ells Rivers, and waters from the Muskeg River. Some of the toxic samples were from sites close to industry (Steepbank River sediments, snow samples close to stacks, Muskeg River waters). Other samples (groundwaters, Ells River waters) showed toxicity far from oil sands activities, with effects in lab fish seen at "background" sites where natural oil sands weathering or water movement through bitumen occurs. Samples were analyzed for naphthenic acids, PAHs, C1-C4 alkylated PAHs, and metals. Sites where sediments and waters were toxic in lab fish bioassays are being assessed to determine whether wild young-of-year fish are abundant and growing normally in these areas of potentially toxic sediments or waters. The results of this work will help guide future studies and locations to sample wild fish and invertebrates to fully assess environmental health in the oil sands area.

Assessing spatio-temporal variations of contaminants in wetlands and wood frogs (*Lithobates sylvaticus*) from the oil sands region of northern Alberta, Canada (PL)

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As part of the Canada-Alberta Joint Oil Sands Monitoring Program, we are investigating wetland health and abiotic and biotic factors of wetlands potentially affected by oil sands industrial development, using 21 wetlands within and outside of oil sands development areas. One goal of the monitoring project is to investigate the spatio-temporal patterns of contaminant levels, including mercury (Hg) and polycyclic aromatic hydrocarbons (PAHs), in wetlands and amphibians. Wood frogs (*Lithobates sylvaticus*) are abundant across the study region and inhabit wetlands downwind of oil sands upgraders where environmental contamination may stem from deposition of airborne contaminants. Parameters being examined include water chemistry, amphibian population biology, and levels of Hg, trace elements, and PAHs in breeding pond water and amphibian tissues. With respect to Hg, across all sites, levels of total Hg in water ranged from 0.34 to 21.3 $ng \cdot L^{-1}$ and did not vary significantly within or across years.

Further, total Hg levels did not exceed CCME limits for the protection of freshwater aquatic life at any of our 21 study sites. To assess Hg levels in amphibian tissues, tadpoles, recent metamorphs and adults were treated separately. The concentration of total mercury was lower in recent metamorphs compared to adults. Across all sites, levels of Hg in tissues ranged from below detection limits to 0.41 $\mu g \cdot g^{-1}$ dry weight. Linear mixed models using current data indicated that variations in the concentration of Hg in frog tissues were not related to distance from upgraders, but were related to the levels of Hg present in breeding pond water, selenium levels in tissues, and water conductivity. Levels of Hg in breeding pond water were also not related to distance from upgraders, but with the levels of zinc and lead present in water as well as water conductivity. Results are being used to design and inform long-term monitoring plans for the oil sands.

Descriptive models for acute toxicity of vanadium to *Daphnia pulex* under different water chemistry conditions representative of surface waters in the Athabasca oil sand region (PO)

Esteban Gillio Meina[1] and Karsten Liber[1]

[1]University of Saskatchewan

Vanadium (V) is abundant in petroleum coke, a by-product of bitumen upgrading in the Alberta oil sands industry, reaching levels of up to 5% V by weight. Annually, coke production at the Alberta oil sands is approximately 8.5 million tonnes, a volume that presents some logistical and environmental challenges. Recently the use of coke as a sorbent to reduce concentrations of organic chemicals in oil sands process water (OSPW) has been explored. Unfortunately, in the process V is released from the coke and can increase in "treated" OSPW to levels as high as 7 mg $V \cdot L^{-1}$; little work has been done to understand how routine water quality variables affect V toxicity to aquatic organisms. Here the potential for adverse effects of V to aquatic organisms is described through the creation of site-specific models for key OSPW water quality characteristics using *Daphnia pulex* as the test species. Preliminary results indicate that a decrease in pH revealed a threshold relationship, where toxicity increased between pH 6 and 7 and then leveled off. An increase in alkalinity (100-600 $mg \cdot L^{-1}$ $CaCO_3$) and sulphate (30 to 300 $mg \cdot L^{-1}$) concentration raised the LC_{50} to *D. pulex* by 0.3 - 0.4 $mg \cdot L^{-1}$, whereas changes in water hardness or chloride concentration showed no modifying effects.

Characterization of oil sands acid-extractable organic fractions using ESI-HRMS (PO)

Anthony Bauer[1], Richard Frank[2], John Headley[2], Kerry Peru[2], L. Mark Hewitt[2] and D. George Dixon[1]

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Alberta, Canada, holds the third largest reserve of oil worldwide next to Saudi Arabia and the largest deposit of oil sands encompassing 140,200 km^2. The waste product of mining, upgrading, and refining oil sands material into a crude oil is a mixture comprised of 70-80% process and/or fresh water, 20-30% solids, and 1-3% unrecovered bitumen. The process material, termed tailings, contains trace metals, high salinity, and dissolved organic compounds. The Alberta *Environmental Protection and Enhancement Act* (AEPEA) prohibits the release of tailings waste into the natural environment and requires that lease sites be remediated to a state capable of sustaining a viable ecosystem. Reclamation strategies include creating aquatic environments in the form of lakes and fen wetlands. The most common approach involves the creation of end-pit lakes, which utilize mined-out pits into which solidified tailings will be deposited and capped with water. The success of this strategy requires the detoxification of tailings and oil sands process water, which has been shown to be achievable through natural biodegradation by indigenous microbes. The contaminant of greatest concern in tailings is widely considered to be the dissolved organic acids. Although these organic acids have previously been considered analogous to naphthenic acids, recent chemical analysis has revealed chemical structures which do not permit this classification.

In this study, five distillate fractions of organic acids extracted from oil sands process water were characterized using electrospray ionization high-resolution mass spectrometry (ESI-HRMS) and synchronous fluorescence spectroscopy (SFS). The five fractions displayed increasing mean molecular weight with increasing distillation temperature: 130°C Fraction, 237 Da; 160°C Fraction, 240 Da; 190°C Fraction, 257 Da; 220°C Fraction, 308 Da; >220°C Fraction, 355 Da. Analysis using ESI-HRMS showed that concurrent with increasing mean molecular weight of each fraction was an increase in the relative abundance of nitrogen-, sulfur-, and oxygen-containing ions and number of double-bond equivalents (DBE). Similarly, analysis with SFS indicated an increase in the degree of aromaticity with increasing fraction mean molecular weight. Analysis of the higher molecular weight fractions (220°C and >220°C) displays compelling evidence for the presence of heteroatomic compounds, dihydroxy/dicarboxyl groups, and compounds that are structurally similar to estrogens. Because natural biodegradation of oil sands organic acids over time displays a proportional abundance of more recalcitrant, higher molecular weight acids, this study is important in the context of tailings remediation.

Developing Critical Effect Sizes as Triggers for Long-term Monitoring Programs

Estimating critical effect sizes and normal ranges using multiple observations from reference sites (PL)

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Determining when a change is an effect is an important challenge in ecotoxicology. Most contemporary studies use small-scale observations and analytical statistics to determine the probability of an impact. A better option is to expand the scope of comparisons beyond the typical upstream-downstream design and define expected ranges (i.e., context) of observations over multiple scales. If we assume that our indicators are sensitive to stress, and we sample sites among multiple spatial and temporal scales (site-specific and compared to local and regional reference sites over time), any changes at an exposed site within the range observed at the scale of interest should not elicit attention or action; anything outside of that range is unusual and would attract attention and further study to confirm. We used many years of sampling white sucker for LSI, GSI, and K in the Moose River basin in northern Ontario to develop expected absolute ranges of normal within a site, compared to local and regional reference sites, and to derive expected relative differences (critical effect sizes; CES) among regional reference sites. The normal range uses previous observations to predict future data. When that future data is collected and is within the expected range, the new data is integrated into the estimate of normal for that site. An exceedance leads to confirmation using a fixed normal range. The expected differences between sites uses data from years in which more than three reference sites were sampled to create an "expected" value (average of three reference sites) compared to an "observed" value (single reference site) to derive relative differences (%). This process was repeated using a unique expected range for each reference site sampled, using the average of all other reference sites sampled within that year. The regional approach described here uses CES and normal range to describe the spatial and temporal context within which exposed sites are found. The approach allows deeper and more comprehensive evaluation of unusual changes (i.e., warnings) that may require further attention and protect all parties from incorrect conclusions.

What were we thinking? Calculating implied critical effect sizes from previously published environmental research (PL)

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Historically, critical effect sizes have rarely been explicitly used to guide the design and interpretation of ecological experiments despite their importance for making well-informed decisions from environmental datasets. However, the very decision to conduct an ecological experiment implies there are potential effects that would be considered important to be able to detect, if they were to exist. The critical effect size implicit in the design and interpretation of an ecological experiment can be quantified by calculating the effect size at which the significance level chosen as the decision-making threshold (typically α=0.05) is equal to the optimal significance level that minimizes the combination of Type I and Type II errors, given the sample size used in the study. This resulting implied critical effect size is the effect size that the ecological experiment was optimally designed to be able to detect, assuming the intent of the experiment is to minimize any errors (i.e., assuming Type I and II errors are equally serious to avoid). If costs of Type I and II errors are known to be unequal, implied critical effect sizes can still be determined by incorporating the relative costs of Type I and II errors into the calculation. Knowing the implied critical effect sizes of previously published research can be useful both as a means of informing future study design and also as a baseline for discussions among stakeholders concerning the determination of explicitly defined critical effect sizes.

Adaptive management in Canada's Northwest Territories: Using prediction intervals as benchmarks to quantify natural variability in environmental monitoring studies (PL)

Rainie Sharpe[1], **Timothy Barrett**[1], **Hilary Machtans**[1], **Peter Chapman**[1] **and Alexandra Hood**[2]

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Aquatic environmental monitoring studies typically quantify biological change by performing statistical tests on relevant endpoints from an exposure area and a reference area. Recent guidance from the Northwest Territories (NWT) Water Boards has encouraged incorporation of an adaptive management approach towards aquatic effects monitoring programs (AEMPs) in the NWT. Adaptive management requires the *a priori* definition of "action levels" and "significance thresholds" which define what level of environmental change will trigger management action, and what threshold of environmental change is deemed unacceptable under any circumstances, respectively. The benefits of incorporating an adaptive management approach to AEMP practice is clear and widely accepted among stakeholders; however, the approach to defining action

level triggers and significance thresholds is widely variable. The successful implementation of adaptive management requires the inclusion of biologically and ecologically meaningful endpoints to serve as action level triggers, but also an understanding of the magnitude of change in these endpoints that are indicative of natural variability versus changes that are due to anthropogenic sources. A novel approach to quantifying natural variability using prediction intervals will be discussed, and a "temporal-reference-normal range" decision matrix towards triggering an action level will be presented.

Lessons from the oil sands on how to interpret invertebrate community effects (PL)

Bruce Kilgour[1], Heather Keith[2] and Kelly Munkittrick[3]

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Environmental effects monitoring (EEM) programs use collections of fish populations and benthic invertebrate communities to assess effects of final treated effluents (pulp and paper, mining) on aquatic receiving environment. Samples of fish and invertebrates from an area exposed to effluent are typically compared to samples of fish and invertebrates from areas unexposed to effluent. The EEM framework for metal mining includes (a) routine monitoring; (b) confirmation of effects (if any); (c) determination of extent and magnitude; and (d) investigation of cause (to determine precise effluent-related causes of biological effects). In the metal mining EEM program, "any" statistically significant difference between reference and exposed samples triggers stage 2 or confirmation, regardless of the nature or biological magnitude of the difference. The spatial designs are highly prone to producing statistical differences, naturally, and there are therefore numerous instances of facilities triggering into IOC stages when the effects are arguably trivially small. Based in part on an analysis of 15 years of benthic invertebrate data collected during monitoring programs associated with the oil sands sector, we illustrate how easily the existing triggers can be exceeded. We also suggest a focusing on temporal variation in the exposure area, to determine first if there is change, second to determine if the change is large, and thirdly to determine if change in the exposure is mimicked by change in nearby reference areas. The change in focus is subtle, but reduces the likelihood of triggering into investigation of cause when there is no need to.

Quantifying natural variability as a method to detect environmental change: Definitions of the normal range (PL)

Tim Barrett[1], Rainie Sharpe[1], Hilary Machtans[1], Peter Chapman[1] and Alexandra Hood[2]

[1]Golder Associates, [2]De Beers Canada

The normal range can be defined as the range that encloses 95% of reference values and can be used as a criterion to assess environmental effects. In practice, the normal range has been defined as the reference mean ± two standard deviations. When sample sizes are low and reference data are not normally distributed, the reference mean ± two standard deviations is a poor estimate of the range that encloses 95% of reference data values. We use prediction intervals to define the normal range of natural variability for a single exposure observation and the mean of an exposure sample of a given size. The prediction intervals treat the reference mean and standard deviation as estimates of the population mean and standard deviation, and incorporate the sample size into the determination of the normal range. The prediction intervals are defined on normally distributed reference data and normality can typically be achieved after data transformation. Covariates can be included in the definition of the normal range to explain some of the variability in the reference distribution and increase ability to detect change. The normal range is a method to quantify natural variability and assess change from reference conditions, and can be used to identify the need for further study.

Industrial Effluents

Environmental regulations: The path from settlement to industrialization (PL)

Gordon Craig[1]

[1]G.R. Craig & Associates

The 1800s were the era of immigration, settlement, harvesting water power, clearing of forests for agriculture and masts for the British Navy, construction of buildings and barns. Tanning operations needed spring hemlock bark, concrete and limestone provided permanence, pulp and paper, gold and iron mines brought wealth and industrialization. Canada's natural resources were expansive and unlimited. Local agricultural and milling activities had no evident impact on the landscape. The cornucopia of riches was there for the taking and it was taken in abundance. The 1900s exhibited the intensification of agriculture, lumbering, industrial processing of food, leather goods, paper production, smelting operations and textiles. The assimilative capacity of local streams and rivers was breached. Surface waters looked dirty, smelled bad, and fish disappeared. The landscape had indeed changed. We will explore the motivation of provincial and federal governments to restore water quality and the challenges they faced with a century old mindset, economic constraints and global unrest. Aquatic toxicology, in its early forms, quantified the degradation of water quality and provided the substance for guideline derivation. Only then did governments have the material to create regulations that would restore the quality of rivers and streams. The 1949 landmark case of the Kalamazoo Vegetable and Parchment Co. in Espanola, Ontario, sets the stage for a revolution in environmental care.

Industrial discharges in Ontario: Past, present and future (PL)

David Poirier[1] **and Lisa Kennedy**[1]

[1]Ontario Ministry of the Environment and Climate Change

Ontario is home to 13 million inhabitants, has 10% of the earth's fresh water and is the most industrialized of all Canadian provinces. This combination increases the possibilities for significant degradation of aquatic ecosystems. In 1987, the Ontario government began discussions with industrial groups for the purpose of implementing province-wide regulations to limit industrial and municipal effluent discharges into the environment. The result was a three-pronged approach called the Municipal/Industrial Strategy for Abatement (MISA), comprising (a) a one-year regulated monitoring program for each of the 10 designated industrial sectors; (b) an engineering survey to identify the best available treatment options (economically achievable) (BATEA) for each sector; and

(c) promulgation of effluent limits regulations (EMEL - Effluent Monitoring, Effluent Limits) for each sector, based on the results of (a) and (b) above. The MISA regulations encompassed over 1000 industrial direct dischargers, and took eight years to complete. The monitoring component of the regulations included analyses for hundreds of different chemical parameters, basic effluent characteristics (e.g., temperature, flow rate) and monthly acute toxicity testing with Rainbow Trout (*Oncorhynchus mykiss*) and *Daphnia magna*. This talk will present a brief summary of acute toxicity testing results from routine MISA monitoring data compared with Ontario Ministry of the Environment audit programs. We will investigate the results from sublethal toxicity tests submitted through the EMEL regulations, identify other ministry programs implemented for pollution control and present ideas for further investigations.

Toxicity reduction and screening of radioactive liquid waste from Ontario's CANDU Stations, an intermittently toxic mixture of variable composition (PL)

Dave Rodgers[1]

[1]Aberfoyle AquaScience and Kinectics

As with other industries, the Electric Power Generation Sector was subject to effluent monitoring and effluent limits under Ontario's Municipal/Industrial Strategy for Abatement regulations. Of the process effluent discharges from Ontario CANDU stations, the Radioactive Liquid Waste System (RWLS) that collects, segregates, monitors, processes and discharges water from numerous drains situated in radioactive zones within the stations was the most difficult, as it was intermittently toxic and varied among stations. Initial studies established that toxicity was correlated with conventional inorganic and organic pollutants rather than radioactivity; however, no single chemical or compound or simple combination of compounds was consistently detected at concentrations sufficient to account for observed toxicity. The focus then shifted to toxicity identification and reduction at individual stations and sources, with an emphasis on utilizing existing radioactivity control process (filtration and selective sorption) rather than new processes requiring extensive alterations. In particular, broadly based treatments were sought to account for future unknown toxicants. Laundry waste emerged as a consistently toxic waste stream and was diverted from the RLWS for treatment at a common facility for all stations. The station with a laundry facility then adopted a reverse osmosis treatment to remove organic contaminants from this waste.

At a second station, hydrazine emerged as the principal component of toxicity, and was treated by oxidation with sodium hypochlorite followed by passage across an activated carbon filter. At the remaining two stations, filtering and sorption processes were upgraded to remove contaminants, especially metals, from the non-laundry waste stream. As toxicity breakthrough remained a potential problem, a short-term screening test, the *Daphnia* IQ (DIQ) test, was used to test RLWS effluent before discharge and very likely prevented release of potentially toxic discharges. However, with subsequent improvements to RLWS treatments, many of which were worked out as a result of this

screening, routine pretesting using the DIQ test was phased out. Ontario CANDU stations now employ Derived Emission Limits (DELs) based on pre-discharge chemical analysis and the results of station specific chemical and toxicological analysis to ensure effluent quality.

Ocean gyres: A possible source of persistent organic pollutions (PL)

Wenhan Cheng[1], Zhouqing Xie[2], Jules Blais[1], Pengfei Zhang[3] and Liguang Sun[2]

[1]University of Ottawa, [2]University of Science and Technology of China, [3]City College of New York

Under the Stockholm Convention, persistent organic pollutions are defined as organic compounds that are toxic, persistent, bioaccumulative, and have potential for long-range environmental transport (LRET). However, most research on LRET has focused on long-range atmospheric transport, with oceanic transport not receiving much attention. For example, it is believed that perfluorooctanoic acid (PFOA) is transported to the Arctic mainly by ocean currents, and the horizontal mixing caused by currents in the western mid-Atlantic could be the major reason of homogenous distribution of polychlorinated biphenyls (PCBs) in that area. Research on enantiomeric composition of hexachlorocyclohexanes (HCHs) indicated a local contribution of 24% HCHs in ocean boundary layers in remote regions. Our results also showed high pollution of organophosphorus esters (OPEs), one of the most common groups of plasticizers, in Prydz Bay of Antarctica, which was remote from any sources of these compounds. As semi-volatile compounds, their lifetime in the atmosphere is not long enough to support long-range transport via atmospheric circulation from source areas in low latitude to Antarctica, thus these compounds should be transported via ocean currents. On the other hand, plastic debris could accumulate in ocean gyres, resulting in high OPE concentrations where plastics accumulate. OPEs are hydrophobic compounds, and they are not chemically bonded to plastic products, making them easier to volatize into the atmosphere. Thus the atmosphere OPE concentration is strongly related to concentration in seawater. Our results showed a positive relationship between concentrations of organophosphorus esters in air and average vorticity of sample area in remote samples, suggesting that high density of plastic debris could result in high OPEs pollution. Several recent studies have revealed that plastic debris could also act as an important vector of contaminants in the marine environment, carrying polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs) and other persistent organic pollutants (POPs). Therefore high density of plastic in ocean gyres may not only lead to high pollution of OPEs, but also POPs.

The persistence and toxicity of hydrazine in freshwater ecosystems (PO)

David Poirier[1] and Richard Chong-Kit[1]

[1]Ontario Ministry of the Environment and Climate Change

Hydrazine (N_2H_4) is a highly reactive inorganic chemical commonly used in rocket fuels, in the production of pesticides and pharmaceuticals, and as an oxygen scavenger in industrial cooling waters in Ontario. Over the past few years, there have been several accidental discharges of hydrazine into surface waters, and the provincial and federal governments wish to understand the potential environmental impacts of this material on aquatic life. Due to its reactive nature, it was thought that hydrazine would be short lived in freshwater ecosystems, but few studies have been conducted. This study looked at (a) the persistence of hydrazine in water at 5°C and 15°C, in the presence and absence of fish; and (b) the acute toxicity of hydrazine to four species of fish and five species of invertebrates. Hydrazine was highly toxic to aquatic animals with 48- or 96-hour LC_{50}s ranging from 0.13 to 17.88 $mg \cdot L^{-1}$.

Changes in environmental attitudes of industry: Past motivation and future direction (PO)

Gordon Craig[1]

[1]G.R. Craig & Associates

In the early days of North America settlement, industry was encouraged by government to utilize natural resources and bring development and wealth to communities. As populations grew and other demands developed, regulations were created to protect natural resources for multiple uses. Industry then argued that environmental regulations increased costs and reduced their ability to compete nationally and internationally. Initially governments protected industry from the costs of environmental controls. It is only over the last 50 years that environmental regulations and legal precedents have placed substantial responsibility for the protection of natural resources on industry. In recent decades, product advertising and marketing has engaged the consumer as another stakeholder in shaping industry's attitude regarding environmental management and protection. The financial model for business has expanded with a growing economy and an increase in stakeholders. Fuller cost accounting incorporating the expense and benefit of environmental protection and remediation has changed the equation of profitability. The imperative for corporations to diligently reduce the probability and severity of environmental damage is now the norm. Corporations and directors who ignore the consequences of environmental impact will be punished by a wider range of stakeholders than ever before — including consumers, investors, regulators, the courts, and competitors.

History of water quality regulations in Canada (PO)

Gordon Craig[1]

[1]G.R. Craig & Associates

The 1800s were the era of immigration, settlement, harvesting water power, clearing of forests for agriculture and masts for the British Navy, construction of buildings and barns. Tanning operations needed spring hemlock bark, concrete and limestone provided permanence, pulp and paper, gold and iron mines brought wealth and industrialization. Canada's natural resources were expansive and unlimited. Local agricultural and milling activities had no evident impact on the landscape. The cornucopia of riches was there for the taking and it was taken in abundance. The 1900s exhibited the intensification of agriculture, lumbering, industrial processing of food, leather goods, paper production, smelting operations and textiles. Industry brought villages, towns and cities with increased urban density. The turn of the 20th century was fraught with disease, infant mortality and epidemics of cholera and typhoid. The assimilative capacity of local streams and rivers was breached. Surface waters looked dirty, smelled bad, and fish disappeared. The landscape had indeed changed. Pressing public health needs led the beginnings of water quality management. The motivation of governments, provincial and federal, to restore water quality faced challenges of a century old mindset, economic constraints and global unrest. Legislation began with governments enabling the development of water quality guidelines, then discharge limits, followed by chemical and toxicity effluent regulations. The rate of regulation development ramped up dramatically after 1975 among the provinces and the federal government. Current water quality regulations have been developed in the life of today's living generations.

Northern Development: Simple Systems, Complex Challenges

How Aboriginal engagement requirements are influencing northern development in the Mackenzie Valley, Northwest Territories (PL)

Sarah Elsasser[1]

[1]Wek'èezhìi Land and Water Board

The Mackenzie Valley, Gwich'in, Sahtu, and Wek'èezhìi Land and Water Boards (the Boards) of the Northwest Territories regulate the use of land and water and the deposit of waste through the issuance of land use permits and water licences. Effective June 1, 2013, the Boards implemented a new Engagement and Consultation Policy that applies to all new applications and submissions. The Policy was developed to ensure that the Boards' obligations for meaningful consultation (as set out by land claims and applicable legislation) are met, and requires pre-submission engagement with affected parties, as well as submission of an engagement plan detailing engagement activities that will be undertaken throughout the life-of-project. The proponent's engagement efforts contribute to involvement of affected parties and are essential in our co-management system. Recent examples of how the Land and Water Boards' new Policy is influencing northern development and water quality-related decisions will be discussed, including implications for the design and reporting of Aquatic Effects Monitoring Programs and Aquatic Response Frameworks.

Considerations when estimating ambient water quality concentrations in the Canadian North (PL)

Barry Zajdlik[1]

[1]Zajdlik & Associates Inc.

Ambient concentrations are defined as natural background concentrations plus contributions from long range transport. There is currently little guidance on estimating ambient water quality concentrations and no guidance that deals with uniquely Northern issues. Determinants of the requisite rigour for an ambient water quality concentration estimate such as uncertainty constraints and decision rules are reviewed. General factors affecting estimation of ambient water quality concentration, such as seasonality, stream order, flow regime, associations with regolith, and population definition are discussed. Natural influences of water quality such as natural acid rock drainage, mineralization and forest fires are also discussed. Finally, the implications of permafrost, thermokarst

degradation, cryoconcentration and long-term ice cover on estimating ambient water quality concentrations are reviewed.

Giant Mine's legacy of arsenic and enhanced mercury methylation in small taiga shield lakes of the Yellowknife, Northwest Territories region (PL)

Adam Houben[1], Rebecca D'Onofrio[1], Steven Kokelj[2] and Jules Blais[1]

[1]University of Ottawa, [2]Aboriginal Affairs and Northern Development Canada

The Giant Mine in Yellowknife, Northwest Territories, operated during 1949-99, is leaving as its legacy 237,000 tonnes of toxic waste arsenic trioxide (As_2O_3) dust, among other compounds, from gold ore extraction and roasting processes. In the past, direct effects of waste leachate have been measured on site in adjacent tailings ponds, ground water, and streams. However, we show here the extended range and lasting impact of roaster-derived emissions on small shallow taiga shield lakes within a 25-km radius of the mine, 11 years after closing. Aquatic arsenic (As) concentrations are well above the 5 $\mu g \cdot L^{-1}$ threshold for the protection of aquatic life and 10 $\mu g \cdot L^{-1}$ guideline for drinking water. In July 2010, As ranged from up to 136 $\mu g \cdot L^{-1}$ in lakes closest to the mine, to 2.0 $\mu g \cdot L^{-1}$ in lakes 24 km away, with an overall median concentration of 8.4 $\mu g \cdot L^{-1}$.

Using the very few historical records of aquatic As concentrations in lakes outside of the mining site, we compared three lakes in 1978 and found similar measurements as a function of distance to the roaster stack. This suggests sustained aquatic As concentrations long after peak mining activity has ended, regardless of several technological advances in emissions reductions over the history of the mine. Suspected roaster emissions also lead to similar observations in our study lakes for related antimony and sulfur compounds. Sulfur dioxide acid rain processes have been cited in the past to be responsible for the degradation of terrestrial vegetation in the immediate area. We also suspect that this elevated sulfate in our study lakes is enhancing mercury (Hg) methylation processes, as the proportion of total mercury (HgT) measured as methyl mercury (MeHg) is exceptionally high in lakes nearest to the roaster stack—up to a 44% MeHg conversion ratio. Though no trends with total or methyl Hg concentrations were observed, we suggest the enhanced Hg methylation efficiency is a result of greater sulfur reducing bacterial activity. This may lead to greater Hg biomagnification in local fish and food webs. These results are of concern, as many lakes of importance to the City of Yellowknife are within this 17-25 km radius of elevated As concentrations and enhanced Hg methylation, including many fishing and recreational lakes. As well, bisecting this area is the Yellowknife River, the city's main source of drinking water, which is classified as "pristine" and subject to limited pre-treatment.

Tracing local and long-range transport of metals to Yellowknife Bay, Great Slave Lake, using lead isotopes (PL)

John Chetelat[1], Yassin Ahmed[2], Brian Cousens[2], Derek Muir[1], Marlene Evans[1] and Murray Richardson[2]

[1]Environment Canada, [2]Carleton University

Yellowknife Bay on Great Slave Lake is a water body of cultural, subsistence and recreational importance for the Yellowknives Dene First Nation and residents of Yellowknife in the Northwest Territories. Metal levels found in Yellowknife Bay reflect the cumulative impacts of human activities at different geographic and temporal scales. Historical metal loadings to the bay occurred during the operation of two gold-bearing mines from 1942 to 2004. Metal deposition may also originate from urban development and the release of aerosols from combustion and dust. In addition, the bay receives atmospheric deposition of metals from the long-range transport of regional and international emissions. This presentation will show preliminary results from the first year of a study on the sources and transport pathways of metals to Yellowknife Bay and the main body of Great Slave Lake. Lead has four stable isotopes (204Pb, 206Pb, 207Pb, 208Pb), and different lead sources have distinct "fingerprints" (ratios of these isotopes) that allow them to be distinguished. We are measuring lead isotopes in various environmental compartments (lichens, terrestrial soils, lake and river sediment, zooplankton, fish) with the objectives of (a) identifying the relative importance of watershed versus atmospheric metal transport; and (b) estimating the contribution of different lead sources to present day bioaccumulation in fish. Sediment core profiles will be examined to reconstruct historical trends in metal deposition to the area. This study on metal sources and transport, using lead isotopes as a tracer, will provide insights into local and long-range influences on a mine-impacted aquatic ecosystem in northern Canada.

Effects of mild to moderate total dissolved solids on a sub-Arctic lake (PL)

Hilary Machtans[1], Tamara Darwish[1], Katherine Harris[1], Peter Chapman[1] and Alexandra Hood[2]

[1]Golder Associates, [2]De Beers Canada Inc.

Studies of mine effluent are traditionally focussed on the effect of metals on aquatic ecosystems. Higher salt concentrations are now being recognized as major components of some minewater, particularly in underground mines. Traditional water treatment plants for mines are not designed to remove salts. As such, numerous mines release low to moderate levels of salts into freshwater systems across Canada. Salinization, at high enough concentrations, can damage freshwater ecosystems. However, little is known about the effects of mild to moderate salt concentrations on freshwater ecosystems, particularly in the sub-Arctic with species that may or may not be

resilient to salts. Snap Lake, a small lake in the Canadian north, has received mild to moderate salt deposition for ten years (2004 to 2014). Total dissolved solids concentrations ranged from approximately 10 $mg \cdot L^{-1}$ at baseline to 350 $mg \cdot L^{-1}$ currently; TDS composition has changed from a carbonate/sulphate ion base to calcium and chloride dominance. Snap Lake presents an opportunity to examine the effects of salinity at modest concentrations with a dominant chloride ion. Chronic toxicity tests, plankton, benthic and fish community studies as well as fish health and fish tissue studies have been conducted over the past ten years. Results indicate the plankton community is changing and fish may be moving toward a more littoral-based food source than at baseline. Fish tissues may be changing in ion composition. Overall, changes at these salt concentrations were observed to be subtle and are confounded by nutrients in the mine effluent, which are causing mild enhanced productivity. Salinization is arguably of increasing concern not just from mines (e.g., agricultural runoff, salinization); continued salinization of Snap Lake provides a case study for assessing effects to northern freshwater communities.

Lake Nipigon baseline study for waterpower development (PO)

Tara George[1], Rick Salmon[2] and Todd Kondrat[1]

[1]Ontario Ministry of the Environment and Climate Change, [2]Ontario Ministry of Natural Resources

Lake Nipigon is the largest lake within the boundaries of Ontario and is an important fisheries and recreational resource. Several significant river systems discharge into Lake Nipigon, some of which have proposed hydroelectric developments as a result of the new *Green Energy Act* and Feed-in-Tariff (FIT) Program and the public demand for more sustainable energy. Currently, the Little Jackfish River (LJF) and the Namewaminikan River, two large systems that discharge to the Lake, have proposed developments. It is well documented that increases in total mercury (THg) and methyl-mercury (MeHg) are a direct result of the establishment of waterpower developments; some types of operations result in more of a release than others. The purpose of this study was to establish a baseline for THg and MeHg in sediment, water, young-of-year (YOY) and sport fish tissue, and benthic invertebrate tissue at the mouths of these two tributaries, as well as the Ombabika River, a tributary without future development. In addition, open water stations in the south and north ends of the Lake were established for comparison. Samples were collected in summer 2012 and analyzed for low-level THg and MeHg, as well as general chemistry and metals. Total mercury and MeHg concentrations in water ranged from 0.33 to 2.13 $ng \cdot L^{-1}$ and 0.01 to 0.10 $ng \cdot L^{-1}$, respectively. Total mercury concentrations in sediment ranged from 2.81 to 57.9 $ng \cdot g^{-1}$ dry weight, with MeHg accounting for an average of 0.3% of the THg concentration. Methyl-mercury did not correlate well to THg or total organic carbon content in the sediment. Levels of THg and MeHg in the water and sediment did not exceed Ontario Ministry of the Environment and Climate Change (MOECC) objectives or guidelines, and were comparable to other non-

impacted systems. However, concentrations of MeHg in the YOY and sport fish tissue collected from the LJF, Namewaminkan, and Ombabika (sport fish only) Rivers did exceed the Canadian tissue residue guideline for the protection of wildlife consumers of aquatic biota (33 $\mu g \cdot kg^{-1}$). The concentrations of THg were below the MOECC fish consumption advisory benchmark for the general and sensitive population. Biomagnification of MeHg was observed through the food chain, with a 9- to 15-fold increase in concentrations from benthos to YOY tissue, and a 3- to 6.5-fold increase in the concentration from YOY tissue to sport fish tissue. Only 5% of the THg concentration in the benthos tissue was comprised of MeHg, while in the YOY tissue, 80% of the THg was MeHg. Overall, the baseline data gathered in this study will be useful in the future for measuring the potential impacts of waterpower developments on the tributaries that flow into Lake Nipigon.

Sampling considerations for assessing water mercury concentrations in remote freshwater systems of northern Canada (PO)

Gretchen Lescord[1]

[1]Laurentian University

The consumption of mercury (Hg), a toxic metal, is a concern for both wildlife and humans, and elevated concentrations of Hg in freshwater and wildlife have been reported in remote regions across Canada. However, working in such remote regions, including Ontario's Far North, poses several severe logistical challenges related to proper collection and storage of water samples with such low level concentrations of Hg. For example, several methods exist for preserving freshwater samples, including filtration for removal of organic matter and freezing to prevent sample degradation; few studies have examined the efficiency and effects of these different sampling methods on Hg determinations. This project, part of a larger study examining the complex cycling of Hg across the Attawapiskat Watershed in Ontario's Far North, compares Hg concentrations in water samples taken using these different methods. Preliminary results indicate significantly lower total mercury (THg) and methylmercury (MeHg) concentrations in filtered water samples when compared to unfiltered samples collected at the same time (difference of ~0.02 $ng \cdot L^{-1}$ for MeHg and ~0.30 $ng \cdot L^{-1}$ for THg). Seasonal variability is also being considered and results suggest that filtered water samples have significantly lower MeHg concentrations in the summer when compared to filtered winter samples taken from the same locations. Analysis is ongoing and future results will also show if sampling locations within a lake (i.e., basin selection and water depth) also impacts MeHg or THg concentrations in water samples taken using these various methods. These results will provide important considerations for future Hg sampling in remote regions, especially given the high pressures from impending resource extraction development across Canada's North.

Environmental Effects Monitoring programs to collect data of relevance for methylmercury exposures and risks to both ecological and human receptors at the Lower Churchill Project, Labrador (PO)

Rob Willis[1], Jackie Wells[2], James McCarthy[3] and Peter Madden[2]

[1]Dillon Consulting, [2]Nalcor Energy Lower Churchill Project, [3]AMEC Environment and Infrastructure

Following its release from the environmental assessment (EA) process in 2012, the Nalcor Energy Lower Churchill Project (LCP) is currently in its construction phase, with hydroelectric power generation expected to commence in 2017. As part of meeting various regulatory conditions and requirements during the EA process and post-EA that relate to methylmercury formation and methylmercury exposures and risks to both ecological and human receptors, the LCP is conducting Environmental Effects Monitoring programs that collect data on a number of parameters which will enable the monitoring and prediction of methylmercury formation in the reservoir and areas downstream of the reservoir, the prediction of human and ecological exposures and risks related to methylmercury, and information on the potential future need for fish or other country food item consumption advisories. This presentation outlines the key components and parameters of the monitoring programs and describes how some of the logistical and other challenges associated with planning and implementing such programs in a northern setting have been or are being addressed.

Aquatic Toxicity Workshop Board of Directors

Results of Aquatic Toxicity Workshop membership survey 2014 (PO)

Rosalie Allen Jarvis[1], David Huebert[2], Gordon Craig[3] and Les Burridge[4]

[1]Environment Canada, [2]Stantec, [3]G.R. Craig & Associates, [4]Burridge Consulting Inc.

For 41 years, the Aquatic Toxicity Workshop (ATW) has served to provide a forum for environmental researchers and managers in Canada to discuss and debate scientific findings, regulatory changes, and testing and data analysis methodologies. After more than two years of engaging ATW members on the future of ATW, the Board of Directors issued an online survey during the period of May 15 – June 11, 2014, to assess the preferred timing and brand of the annual workshop. Fifty-three percent of the 224 survey respondents — representing government (76), consulting (57), academia (49), industry (20), students (16) and retired (5) "sectors" — had attended an ATW in the previous three years. Sixty-five percent of respondents would prefer to attend ATW during its current annual timing of early October, as compared with the identified alternative of mid-February. Across all sectors, the majority of respondents preferred the status quo for annual timing. Among those who had not attended ATW in the previous three years, 56% preferred the October timeframe. In addition, 75% of respondents would prefer to change ATW's operating name to reflect an expanded scope that includes ecotoxicity issues beyond a purely aquatic realm. Overall, 57% of respondents preferred the name "Canadian Ecotoxicology Workshop", surpassing support for "Ecotox Canada" (18%) or the status quo (25%). Across all sectors, the majority of respondents preferred the "Canadian Ecotoxicology Workshop" brand. The Board of Directors will announce its decisions on the issues of timing and branding at the Annual General Meeting of members in Ottawa on October 1, 2014.

Update from the ATW Board of Directors

At ATW 2014 in Ottawa, the Board of Directors announced its decision to formally expand the scope of scientific topics addressed at future workshops, based on the views of a majority of ATW survey respondents, and adopted a new workshop name to reflect this decision. On October 1, 2014, the Aquatic Toxicity Workshop changed its name to the Canadian Ecotoxicity Workshop (CEW). Future workshops will provide the opportunity to share information on current and emerging topics of regional, national and international importance related to effects of chemical, biological, and physical stressors on aquatic biota, terrestrial organisms and ecosystems. The goal of this expanded scope is to engage a broader range of professionals involved in various aspects of environmental science in debate and discussion on evolving issues of importance in Canada and elsewhere.

As a complement to the more traditional sessions on aquatic toxicology and ecology, future workshops will have platform/poster sessions, panel discussions and debates on topics such as:

- Method development and ecological research for wildlife ecotoxicology
- Method development and application of sediment and soil toxicity tests
- Amphibians as models in ecotoxicological risk assessment
- Northern development activities and environmental challenges
- Biological effects of data-poor metals and metal mixtures in various environmental media
- Nanotoxicology: environmental fate, transformation and effects on aquatic and terrestrial ecosystems
- Unconventional oil and gas issues, as they relate to aquatic and terrestrial ecosystems
- Risk assessment and the nuclear fuel cycle
- Adverse outcome pathways: linking toxicity-related molecular and cellular responses to ecologically-relevant endpoints
- Development and validation of omics tools for predictive ecotoxicology
- Behavioural measurement and analysis in aquatic and terrestrial ecotoxicology research
- Effects of toxic substances or stressors on populations or ecosystems.

Members and potential members alike are encouraged to follow CEW's presence on social media (Facebook, Twitter, LinkedIn), as well as the workshop's dedicated website (ecotoxcan.ca).

Rosalie Allen Jarvis, Gordon Craig, Guy Gilron, David Huebert and Lisa Taylor

Board of Directors
Canadian Ecotoxicity Workshop

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