Proceedings of the 43rd Annual Canadian Ecotoxicity Workshop: September 25-28, 2016, Edmonton, Alberta

Comptes rendus du 43e atelier canadien annuel sur l'écotoxicité : du 25 au 28 septembre 2016, Edmonton, Alberta

Editors

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Canadian Technical Report of Fisheries and Aquatic Sciences 3218

Rapport technique canadien des sciences halieutiques et aquatiques 3218

Canadian Technical Report of Fisheries and Aquatic Sciences

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Numbers 1-456 in this series were issued as Technical Reports of the Fisheries Research Board of Canada. Numbers 457-714 were issued as Department of the Environment, Fisheries and Marine Service, Research and Development Directorate Technical Reports. Numbers 715-924 were issued as Department of Fisheries and Environment, Fisheries and Marine Service Technical Reports. The current series name was changed with report number 925.

Rapport technique canadien des sciences halieutiques et aquatiques

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 rapports techniques sont produits à l'échelon régional, mais numérotés à l'échelon national. Les demandes de rapports seront satisfaites par l'établissement auteur dont le nom figure sur la couverture et la page du titre.

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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2017

PROCEEDINGS OF THE 43RD ANNUAL CANADIAN ECOTOXICITY WORKSHOP: SEPTEMBER 25-28, 2016, EDMONTON, ALBERTA

Editors:

K.I. Racher[1], M.A. Wilson[2], P.L. Siwik[2], C. Dubetz[3] and R. Allen Jarvis[4]

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Preface

For 41 years, the annual Aquatic Toxicity Workshop (ATW) has been held in various locations across Canada. In 2015, the ATW was rebranded as the annual Canadian Ecotoxicity Workshop (CEW) to reflect the broad scope of environmental interests held by workshop participants.

The 43rd annual CEW was held at the Shaw Conference Centre in Edmonton, Alberta, from September 25 to 28, 2016. The workshop included 149 platform presentations and 43 poster presentations. Total attendance was 293.

This workshop was one of a continuing series of annual workshops in Canada on ecological toxicology, covering topics from basic ecotoxicology 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 ecotoxicology. 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

Pendant 41 années, l'Atelier annuel sur la toxicité aquatique (ATW) a eu lieu à divers endroits autour du Canada. En 2015, l'atelier a été rebaptisé l'Atelier canadien annuel sur l'écotoxicité (CEW) pour tenir compte de l'étendue des intérêts environnementaux généraux des participants à l'atelier.

Le 43ième Atelier canadien annuel sur l'écotoxicité a eu lieu au Centre des congrès Shaw à Edmonton (Alberta), du 25 au 28 septembre 2016. L'atelier a donné lieu à 149 présentations orales et à 43 présentations par affiche. Deux cent quatre-vingt-treize personnes ont assisté à l'atelier.

L'atelier a permis de poursuivre les discussions tenues annuellement au Canada sur 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 écotoxicologie, 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 de Pêches et Océans Canada.

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. Email addresses of lead authors have been included where permission was granted. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

The editors would like to thank Karoliina Munter, Grant Schroeder and Dr. Jill Watson for their 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. Les adresses courriels des auteurs principaux sont incluses si la permission a été accordée. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

Les rédacteurs voudraient remercier Karoliina Munter, Grant Schroeder et Dre. Jill Watson dans la préparation de ces comptes rendus.

43rd Canadian Ecotoxicity Workshop Organizing Committee / Comité organisateur du 43e atelier canadien sur l'écotoxicité

Workshop Co-Chairs / Co-présidentes de l'atelier:

Kathleen Racher	K. Racher Consulting
Paula Siwik	Environment and Climate Change Canada
Anne Wilson	Environment and Climate Change Canada

Workshop Organizing Committee / Comité organisateur de l'atelier:

Ingrid Carleton-Dodds	Consultant
Lindsey Cymbalisty	Mackenzie Valley Land and Water Board
Catherine Evans	Alberta Energy Regulator
Amy Gainer	University of Saskatchewan
Esteban Gillio Meina	University of Saskatchewan
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Greg Goss	University of Alberta
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PO – Poster presentation

Dr. Richard C. Playle Awards for Outstanding Theses in Ecotoxicology

The 2016 winner of the MSc thesis award was Anita Massé, University of Saskatchewan. No BSc Honours thesis award was awarded in 2016.

Reproductive and developmental effects of elevated maternal dietary selenium in the model amphibian *Xenopus laevis* (PL)

Anita J. Massé[1], Jorgelina R. Muscatello[2], Natacha S. Hogan[1] and David Janz[1]

[1]University of Saskatchewan, [2]Stantec Consulting Ltd.

Selenium (Se) is a contaminant of potential concern in aquatic systems 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 in fishes and birds have been the primary focus of Se research thus far, while studies focusing on Se toxicity in amphibians are severely lacking. A generational bioassay was performed to evaluate the effects of elevated *in ovo* Se exposure via maternal transfer on early and late stage tadpole development in addition to the overall fitness of adult *Xenopus laevis* females after a chronic dietary exposure to increasing concentrations of L-selenomethionine (Se-Met). The results revealed elevated *in ovo* Se exposure increased the frequency and severity of morphological abnormalities during early tadpole development with minimal effect on hatchability or mortality. Late stage tadpole survival and growth was unaffected by *in ovo* Se exposure; however, those in the highest dose group were more likely to be at earlier stages of development despite no difference in time to metamorphosis. In addition, endpoints related to adult female fitness showed no significant differences among treatment groups. *X. laevis* has increased tolerance to elevated levels of *in ovo* and dietary Se when compared to other oviparous vertebrates studied to date; however, further research is necessary to determine whether *X. laevis* is a tolerant, average or sensitive species among amphibians.

Invited Plenary Speakers

Planning for uncertainty using the past: Paleo-ecotoxicological perspectives on aquatic ecosystems (PL)

John P. Smol[1] *and Jules Blais*[2]

[1]*Queen's University* [2]*University of Ottawa*

One of the greatest challenges faced by ecologists, water quality managers, and other environmental scientists is using appropriate time scales to assess environmental change. Due to the lack of systematic long-term monitoring data, it is often difficult to determine the nature and timing of ecosystem changes. This presentation summarizes recent developments in assessing the effects of multiple stressors from primarily resource development on lake ecosystems using sediments as archives. Here, we focus on integrating the principles of ecotoxicology with lake sediment analyses in our work to develop the field of "paleo-ecotoxicology". We argue that this framework is useful to test predictions from laboratory bioassays in natural ecosystems, to disentangle the effects of multiple interacting stressors, as well as to assess the importance of direct and indirect effects for mediating biotic responses to contaminant exposure. Specific examples include ongoing work assessing the long-term limnological impacts of gold mining in the Yellowknife region to the effects of industrial oil sands activities in northern Alberta. The problems of contaminant transport and the over-riding effects of recent climatic change will be highlighted, including the challenges faced when dealing with multiple stressors.

Styrofoam and plastic biodegradation: Good news! (PL)

Peter Heule[1]

[1]*Alberta Royal Museum*

Two materials formerly believed to be non-biodegradable and ubiquitous in consumer and construction waste can be broken down by the common mealworm beetle and naturally occurring bacteria, respectively. Plastic water bottles and Styrofoam are not as permanent as we thought: some bacteria are able to reduce them into less persistent constituents, giving hope for reducing the amount of plastic pollution in the environment and our landfills. The implications for further research and development of biodegradation techniques for problematic pollutants using insects and bacteria are discussed.

Alberta's past environments: A record of thriving inland seas and their eventual demise (PL)

Hilary Corlett[1], *Tyler Hauk*[1] *and Tiffany Playter*[1]

[1]*Alberta Geological Survey*

Three geologists from the Alberta Geological Survey (AGS) will be taking us on a trip through Alberta's distant past and show how Alberta went from being a beautiful tropical paradise to the still beautiful but less tropical state it is in today. Welcome to the Paleozoic Era of Alberta, covering approximately 300 Ma years of geological history. During much of this time, Alberta was covered by a

shallow inland seaway that resulted in tropical reef deposition in numerous sub basins. There were several large extinctions throughout the Paleozoic Era. This presentation will focus on some of the larger ones, including the Permian-Triassic extinction that is known to be the largest mass extinction event in the Earth's history.

25th Anniversary of Canadian Environmental Effects Monitoring

The development of Environmental Effects Monitoring: The early years (PL)

Gordon Craig[1]

[1]*G.R. Craig & Associates*

Regulatory control of the impact of industrial discharges on receiving waters began in the early 1950s with some narrative prohibitions and some quantitative chemical limits. A good example is the 1952 International Joint Commission (IJC) Boundary Waters Objective. The provinces developed their respective physical and chemical effluent limits expressed as concentrations and loads. In the 1960s government regulators and universities conducted benthic surveys downstream of discharges and some industries did the same either voluntarily or under order. The two approaches of point in time end-of-the-pipe effluent quality and integrative benthic and fisheries community assessments continued along separate paths for the next two decades. By the mid-1980s, scientists and managers with Environment Canada and Fisheries and Oceans Canada realized that the two needed to be connected to quantify the impact of industrial discharges on the biological and physical environment. Overriding statutes had always claimed a requirement to protect fisheries and aquatic life but there was little proof that existing controls accomplished that purpose. Historical studies were so varied that spatial and temporal comparisons were difficult to impossible. Workshops were held to review pilot studies in the late 1980s; a strategic plan was developed to meet stated objectives; technical guidance was developed in 1991 to ensure conformity; the industry and consultants critiqued the program; and in 1992, the Environmental Effects Monitoring (EEM) program was created. The objectives and capabilities of the program were debated before and long after promulgation of the regulation. Those early days were a fiery groundswell shift in environmental impact assessment. Everything changed.

A history of national assessment findings from Canada's Environment Effects Monitoring regulatory requirements (PL)

Richard Lowell[1] *and Sylvie Richard*[1]

[1]*Environment and Climate Change Canada*

To investigate the nature and degree of effluent effects on receiving water biota since the 1990s, a series of national assessments has been conducted on Environmental Effects Monitoring (EEM) data submitted under the *Pulp and Paper Effluent Regulations* (PPER) and *Metal Mining Effluent Regulations* (MMER). These assessments have provided a great deal of insight into effluent effects (when they occur), facilitating subsequent investigations of cause (IOC) and solutions (IOS). In areas exposed to pulp and paper mill effluent, the national assessments have shown national average response patterns typically associated with nutrient enrichment/eutrophication effects on fish and invertebrates, together with metabolic disruption (reduced gonad size) in fish. Pulp and paper standard monitoring findings led to IOC and IOS studies that revealed, among other things, the importance of reducing organics loading to help ameliorate both eutrophication and fish gonadal effects. In areas exposed to metal mine effluent, the national assessments have shown a greater heterogeneity of effects (sometimes inhibitory, sometimes stimulatory) among mines, although effects were fairly repeatable from one study to the next for individual mines. Metal mining IOC studies, to date, have shown that the effluent substances associated with effects include major ions, metals, nitrogen, total suspended solids, phosphorus, and selenium. This

long-term series of EEM studies and assessments (for both PPER and MMER) has revealed a wealth of information that was not available before the national implementation of EEM in Canada, and has played a central role in evaluating the effectiveness of current regulations in protecting fish, fish habitat, and fisheries resources.

Science in support of Environment Effects Monitoring over the years (PL)

Mark McMaster[1], Kelly Munkittrick[2], Simon Courtenay[3] and Richard Lowell[1]

[1]Environment and Climate Change Canada, [2]COSIA, [3]Canadian Water Network

During the late 1980s, studies found reduced gonad sizes, delayed maturation and changes in secondary sex characteristics in fish near effluent discharges from some Canadian pulp mills. The findings led to the development of a national, standardized, cyclical monitoring program for fish and benthic invertebrate communities focused on the more than 130 Canadian mills discharging effluent in the early 1990s. This program was expanded to include metal mines with liquid effluent discharges a decade later. Science played a driving role in the development and improvement of Environment Effects Monitoring (EEM) over the years, and is an excellent example of how science and policy can work well together. The industry-funded cyclical program is tiered and adaptive, and decisions on subsequent monitoring depend on the sizes of effects and consistency of patterns of responses. Science-driven expert working groups were formed to provide advice on sample design and data interpretation, and to review results and make recommendations to improve the requirements. A series of workshops, conferences and special issues focused on evolution of sampling methods, design challenges and data interpretation, and a series of science-driven guidance changes focused on improving the monitoring program. Science played a number of globally unique roles in the development of this national program, including developing methods for determining critical effect sizes, setting standardized alpha and power levels for pre-design, scheduling species-specific sampling times, and developing and applying methods for calculating the magnitude of difference on a national basis, in an environment open to multi-stakeholder input and debate. This presentation will review the science development over the years which contributed to the success of the program.

Environmental Effects Monitoring and sublethal toxicity testing: A 25-year symbiotic relationship (PL)

Rick Scroggins[1], Lisa Taylor[1] and Leana Van der Vliet[1]

[1]Environment and Climate Change Canada

Sublethal toxicity testing (SLT) has been a component of the Environment Effects Monitoring (EEM) program under the *Pulp and Paper Effluent Regulations* since 1992. SLT became a requirement for Canadian mining operations through the 2002 amendment of the *Metal Mining Effluent Regulations*, which introduced EEM studies as a requirement. Mills and mines must complete SLT testing using a battery of three or four tests with representative fish, invertebrate, algal and aquatic plant species at a frequency of once or twice per year. In this presentation, we will provide an overview on the application of SLT within EEM. Specifically, the common uses of SLT data will be summarized; how the EEM program requirements have enhanced the capabilities of the Canadian toxicology laboratories and driven improvements in test methods; and a discussion of recent and proposed changes to the sublethal testing requirements. Audience members new to EEM will gain an understanding of the value of laboratory

toxicity tests conducted on end-of-pipe effluents from a regulatory standpoint, while the EEM old timers will be able to relive the ups and downs of this often controversial program requirement.

Dredging up the past: The confounding effects of non-mill activities on the Environmental Effects Monitoring program of Irving Paper Limited, Saint John, New Brunswick (PL)

Tim Vickers[1] and Mary Murdoch[1]

[1]Stantec Consulting Ltd.

Understanding the potential influence of non-mill activities on the characteristics of a receiving environment is critical to an Environmental Effects Monitoring (EEM) program. Irving Paper Limited (IPL) operates a thermo-mechanical refining paper mill in Saint John, New Brunswick, which began discharging secondary treated effluent into Courtenay Bay in 1995. IPL began its EEM Program in 1993, prior to the discharge of effluent into Courtenay Bay. The initial EEM demonstrated baseline environmental conditions characterized by an impoverished benthic invertebrate community (BIC), low and evenly distributed sediment total organic carbon (TOC) levels, and a lack of suitable sentinel species for an Adult Fish Survey (AFS). Field studies conducted in subsequent EEMs programs found BIC composition and sediment quality decreased near the mill outfall. Environment Canada (EC) confirmed a prioritized effect (species richness) for BIC that exceeded the critical effects size (CES) over two consecutive cycles, and indicated that these BIC effects were likely related to the deposition and degradation of carbon rich sediments. The confirmation of prioritized effects for BIC across two consecutive cycles allowed for an Investigation of Cause/Investigation of Solutions (IOC/IOS). Questions remained about the potential for non-mill related influences to confound the interpretation of results. These influences included petroleum and polycyclic aromatic hydrocarbons (PAHs) in the sediments, more than two centuries of untreated municipal wastewater discharges and, perhaps most importantly, the annual dredging of sediments in Courtenay Bay to allow for commercial vessel traffic. Physical, chemical and biological information collected in the first three EEM programs were re-examined in light of third-party information on non-mill factors. The conclusion reached was that there was low potential for municipal wastewater or hydrocarbons to explain the reduction in BIC composition at sampling stations located near to the mill outfall. Although dredging removed sediments annually along the sampling gradient, there remained a distinct pattern of effects to BIC at stations in close proximity to the IPL mill outfall. Overall, the IOC results confirm that the changes in abundance and structure of the BIC close to the mill outfall is most likely related to increased organic deposition from the discharge of mill effluent. Recommendations on future BIC study designs are made to provide additional information to aid in interpreting results. This study highlights how a re-examination of historical information can improve our understanding of EEM results for mills situated in dynamic and industrial urban receiving environments.

Where do we go from here? Lessons from 25 years of Environmental Effects Monitoring in Canada (PL)

Tim Arciszewski[1] and Kelly Munkittrick[1]

[1]COSIA

Monitoring shouldn't be difficult, but it is. And despite the difficulty of detecting and attributing cause, its importance has not diminished. A federal Environmental Effects Monitoring (EEM) program

was developed in the early 1990s for determining the efficacy of guidelines regulating the discharge of pulp mill effluents and (later) metal mines. After 25 years of developing consistent monitoring for large industries in Canada, many lessons can be gleaned for application in other areas. Although implicitly included in the development of EEM, Adaptive Monitoring has recently been formalized and offers an opportunity to develop and operate meaningful monitoring programs. An Adaptive Monitoring program is characterized by hypothesis-driven questions linked to conceptual effect pathways, evolving through trigger-driven feedback loops that connect tiers of monitoring. Triggers describe background variability and notify us when an unexpected change has occurred. Tiers represent an escalation of effort or attention towards those unexpected observations. Tiering allows the simultaneous operation of both broad non-specific tools and targeted sampling. Other recent improvements include the development of adverse outcome pathways (AOP) as simple expected effects pathways. A logical question in an AOP process as applied in monitoring is: if we see a change in some physiological indicator, and the difference matters, what will change next? These ideas and a more detailed discussion on improving environmental monitoring will be presented.

Review of the environmental research funded by the Canadian pulp and paper industry in response to the Environmental Effects Monitoring program (PL)

Pierre Martel[1]

[1]*FPInnovations*

This review will focus on the environmental research funded at FPInnovations (formerly the Pulp and Paper Research Institute of Canada) in response to the Environmental Effects Monitoring (EEM) regulations. Before the first cycle of the EEM program was completed, the need to understand the relationships between process, effluent chemistry and chronic effects in fish was fully recognized. Field studies reported that measurements of somatic indices, mixed function oxidase (MFO) enzyme activity and circulating steroid hormones were symptoms of endocrine disruption. Chlorinated dioxins and furans were suspected as causal agents. In our laboratory, full life cycle tests with fathead minnows (*Pimephales promelas*) found that a 2.5% concentration of bleached kraft mill effluent caused lower egg production and an increased number of individuals with male secondary characteristics. Later, substitution of elemental chlorine for chlorine dioxide in bleaching with improved biological treatment abated the effects. In the late 1990s we gained further insight into the benefits of biological treatment with respect to MFO induction and identified two natural wood extractives as causative agents. Our involvement in field studies in the late 1990s included sentinel species surveys, fish population surveys and the evaluation of caged bivalves. By Cycle 3, accumulated data demonstrated the presence of effects on fish reproduction based on the indicator of reduced gonad size. A Smart Regulation initiative recognized that there was an opportunity to expand the EEM beyond monitoring and address priority issues. Its recommendations included allowing flexibility with the design of investigation of cause studies and that government collaborate with industry to identify the cause of effluent effects on fish gonads. These recommendations were acted upon and a National Project was formed where industry, government and academia joined in a collaborative study. The study plan was supported by the EEM National Office and measures were taken to facilitate mill participation and funding of the project in EEM Cycles 4, 5 and 6. The National Project team first worked to select biological and analytical tools based on lab and field results. Based on these findings, a large-scale effluent survey was proposed in Cycle 6. The survey included 20 mills and examined 81 effluents using chemical markers and a 5-day version of the adult fathead minnow egg production test. One major finding was that effluent organic loading could be correlated with inhibition of egg production for most kraft and thermomechanical pulp mill effluents.

Minimizing organic losses in the process and optimizing biological treatment were proposed as solutions. In future EEM cycles, there will be an opportunity to evaluate how the strategies adopted by mills translate into measurable improvements in the field.

Investigation of cause: A multi-cycle review of contributions to pulp and paper Environmental Effects Monitoring (PL)

Deborah MacLatchy[1], Tibor Kovacs[2], Pierre Martel[2], Mark McMaster[3], Brian O'Connor[2], Joanne Parrott[3], Michael van den Heuvel[4], Glen Van Der Kraak[5] and Mark Hewitt[3]

[1]Wilfrid Laurier University, [2]FPInnovations, [3]Environment and Climate Change Canada, [4]University of Prince Edward Island, [5]University of Guelph

A national assessment of the fish data from the first five cycles of the Environmental Effects Monitoring (EEM) program for pulp and paper identified evidence of enrichment as condition factors and liver sizes of the effluent-exposed fish were larger. This was accompanied by metabolic disruption, a term used to describe impacts on reproductive capacity, defined by the occurrence of larger fish with smaller gonads. Prior to the national assessment, a number of mills were proactively working through Investigation of Cause (IOC) and Investigation of Solution (IOS) studies as prescribed by the EEM program. Irving Pulp & Paper, Ltd. (IPP) in Saint John, New Brunswick, provides a case study for conducting IOC/IOS studies as augmented through the development of additional research and assessment tools. At IPP, the use of mesocosm, laboratory reproductive bioassays for the estuarine fish species mummichog (*Fundulus heteroclitus*), and chemical separation and compound identification studies, through multiple cycles, showed recovery condensates to be a contributor to reproductive endocrine effects. In-mill reverse osmosis treatment of the 5th effect condensates was also shown to be an effective solution. To address on a wider scale the emerging conclusions of metabolic disruption, during Cycle 4 a national research project involving the pooling of resources from Environment Canada, FPInnovations, academia and industry was formed. Beginning with a thorough literature review, the selection of a reliable lab tool for assessing the potential effects of mill effluents on fish reproduction (adult fathead minnow, *Pimephales promelas*), and preliminary studies, the test was applied to various mills through several cycles. Effluents were also subjected to chemical characterization involving a range of parameters, demonstrating that organic loss control is an important component of a strategy to abate potential effluent-related effects on fish reproduction. In particular, measurement of biochemical oxygen demand (BOD) and gas chromatography/mass spectrometry profiles were good indicators of organic losses due to spills, upset conditions or underperforming effluent biological treatment. The approach was also expanded to include assessments of effluent variability at individual mills and confirmed that reducing the loading of organics in the final effluent appears to provide the greatest probability of abating effects on the reproduction of fish in lab tests. Overall, individual mill studies and the national project work done through the auspices of the EEM program indicate that improvements in effluent quality are achievable through reduction of BOD throughout the process; optimization of treatment processes within the mill or through a biological treatment plant; and spill minimization. To that end, the cyclical EEM program has been a successful and responsive regulatory program to support improvements in the environmental effects of pulp mills.

History of using the two-standard deviation normal range as a generic effect-size criterion in Environmental Effects Monitoring (PL)

Bruce Kilgour[1] and Keith Somers[2]

[1]Kilgour & Associates Ltd., [2]University of Toronto

When we were developing the benthic monitoring protocols for the federal pulp and paper Environmental Effects Monitoring (EEM) program, we wrestled with critical effect sizes in order to justify sample sizes. Power analyses and sample size were topics of the day, but the notion of specifying a critical effect size for a benthos monitoring program was a relatively new challenge. The proposal to use the normal range was one of the products from a "pivotal" meeting at Toronto's Novotel in 1992 where government (principally staff from the Department of Fisheries and Oceans, with their consultants) and industry (and their consultants) met to discuss generic approaches to EEM study design. The proposed idea was that environmental effects that exceeded the normal range of variation (i.e., mean ± 2 standard deviations) of reference communities were potentially informative. The concept looked relatively straightforward at first glance, but the statistics for testing that an observed effect exceeds the normal range took some time to sort out, and even longer to gain general acceptance. Although the 2 standard deviation normal range has been criticized as being too statistical and not ecologically meaningful, there has yet to be a different idea that is more meaningful or useful for designing and interpreting benthos monitoring programs.

How before-after-control-impact (BACI) designs can improve biological monitoring for mines under Canada's Environmental Effects Monitoring programs (PL)

Brian Pyper[1], Ryan Hill[1] and Gary Mann[1]

[1]Azimuth Consulting Group

Biological monitoring studies downstream of mine effluent discharges are required as part of federal Environmental Effects Monitoring (EEM) programs. Study designs and initial studies for EEM are not required prior to the commencement of effluent discharges. Consequently, many EEM programs do not have useful data for the period "before" mining begins. Not surprisingly, the experimental design for almost all EEM programs relies on comparison of a potential "impact" (i.e., exposed) site to one or more unexposed "control" sites, or to reference conditions or "normal" ranges. However, there may be large pre-existing differences among sites, and hence, a potential weakness of these designs is the underlying assumption that observed differences (or lack thereof) between "impact" and reference areas can be considered an effect (or lack thereof). Conceptually, a before-after-control-impact (BACI) design is preferable because it can account for natural differences among sites, as well as natural changes over time. In the BACI framework, the effect of interest is a change (from before to after period) in the difference between an impact site and control site(s). Using data for benthic invertebrate community abundance and richness from several locations in Canada, we evaluated the expected performance of control-impact designs and BACI designs. First, we characterized the relative magnitude of natural variation attributable to time, sites, and replicate samples. Second, we used these components of variation to guide simulations that evaluated the Type I and Type II error rates associated with alternative designs. We draw conclusions about when BACI designs are likely to be preferable to control-impact designs, given typical variation in data for benthic invertebrate community abundance and richness.

Environmental effects monitoring development in Brazil (PL)

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The Brazilian Program for Assessment of Water Quality has not yet established a standardized methodology for environmental effects monitoring. Recent movements have shown the importance of integrating physical, chemical, and biological results for environmental health studies. In Canada, this type of monitoring was implemented in the mid-1990s, as part of the federal Environmental Effects Monitoring (EEM) program. The present study aimed at validating and adapting the Canadian EEM program in a pilot project in Brazilian estuarine ecosystems, referred to as the Fish Guide Project. The EEM approach was used to assess the health of three estuaries of Espírito Santo State: the Benevente, Jucu and Santa Maria da Vitória rivers. Three sampling points were selected in each river along a hypothesized contamination gradient. Fish and benthos studies were conducted during three sampling periods in 2014 and 2015 in different seasons (dry and wet). In parallel, water and sediment chemical parameters were analyzed. Three different fish species were chosen as bioindicators, *Genidens genidens*, *Ophioscion punctatissius* and *Eleotris pisoni*. The fish index (GSI, HIS and K) and benthic community structure results showed that the greatest environmental effects were observed in sampling points located in the region next to the sewage discharge, reflecting the results of chemical analyses of water, sediment, and fish filets. From this study, it was possible to evaluate the health status of the three Brazilian estuaries and to publish the Brazilian environmental effects monitoring protocol in December 2015. Adopting this protocol is expected to maximize the effectiveness of environmental monitoring studies while reducing time and cost.

Seven Environmental Effects Monitoring cycles of learning and action at Grande Prairie (PL)

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Since its inception in 1993, the federal Environmental Effects Monitoring (EEM) process for the Weyerhaeuser pulp mill at Grande Prairie, Alberta has successfully identified and quantified mill influences on aquatic receiving environments, implemented innovative investigations of cause in collaboration with government and academic researchers (including distinguishing additive and synergistic effects of effluent discharges by other river users), identified and implemented engineering and technical solutions to address documented effects (including modelling of their potential outcomes), and assessed in the field the effectiveness of these engineering solutions once applied. The history and progression of the Grande Prairie EEM program over time will be presented, with discussion of the limitations of the current EEM process to guide future monitoring at facilities like Grande Prairie that have progressed through all tiers of the EEM investigative and mitigation framework.

Response of biological communities to pulp mill effluent treatment upgrades (PL)

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A southern British Columbia pulp mill which has been operational since 1968 has discharged mill process effluent to an adjacent river system, which is considered a high-gradient, cold water-receiving environment. Since the mill began discharging, monitoring of the receiving environment has shown an eutrophication response. After the completion of the Cycle 4 Environmental Effects Monitoring (EEM) program, it was determined that the mill should go into an investigation of cause for Cycle 5. The cause of the enrichment was determined to be primarily nutrient based, which led to the mill implementing nutrient control strategies during Cycle 6. The Cycle 7 EEM program in 2015 included the first field program undertaken since Cycle 4 in 2006. The results observed during Cycle 7 were clearly positive; whereby effects in the nearfield area were not only less than observed during Cycle 4 but were below Environment Canada's critical effect sizes. The benthic community in the nearfield during Cycle 7 exhibited higher density, richness, and Bray-Curtis dissimilarity index compared to the reference, although the magnitude of difference between the two locations was less in Cycle 7 than in Cycle 4 for these endpoints. In Cycle 4, where statistical differences in endpoints were measured, the differences all exceeded the 3 reference standard deviations, whereas in Cycle 7 the differences were all less than 2 reference standard deviations. Torrent sculpin (*Cottus rhotheus*) populations were measured during both the Cycle 4 and Cycle 7 EEM programs. In Cycle 4, fish results had magnitudes above critical effect sizes for liver weight to body weight in female and immature populations of the near-field compared to reference. In Cycle 7, the near-field results were larger for this endpoint compared to both references but at magnitudes below critical effect sizes. Minor enrichment effects were seen in Cycle 4 for this pulp mill, and changes to treatment were implemented. After nearly 10 years, minor enrichment effects were still observed, but with smaller magnitudes and, in some cases, a small enough difference to be considered not ecologically significant. The EEM program has assisted mills in identifying environmental impairment and provided a process to improve effluent quality so as to reduce effects on the aquatic receiving environment.

Recovery of the intertidal communities at sediment contaminated sites in British Columbia: 25+ years of monitoring data analysis and modelling (PL)

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[1]*Hemmera*

Marine biological communities in British Columbia near historic pulp mills have been recovering over the last two decades since initiation of more stringent enforcement of environmental standards. Improvement in effluent treatment, mill closure, remediation and restoration programs has led to the reduction of contamination loading to the marine environment. Intertidal community monitoring, which has been conducted along pollution gradients in several pulp mill areas from Prince Rupert to Howe Sound since 1990, has documented some recovery of intertidal species diversity and abundance. However, the recovery at sites heavily impacted by industrial effluents and historically accumulated marine depositions was minimal compared to the recovery of the less impacted sites. Data collected since 1990 at several sites located along contamination gradient were analyzed with the following questions in mind: (a) What are the dynamics of recovery at the sites with different impact level? (b) What are the major factors impairing recovery? (d) What are the key species that could facilitate the

successional recovery at the site? (e) Can we accelerate the recovery of intertidal communities through physical and biological habitat enhancement? The results of the analysis and data modelling are presented to illustrate recovery of marine communities in multi-stressor environments, and they can be applied to develop management to facilitate successful marine ecosystem recovery post-pollution abatement.

A novel approach to assess site fidelity and movement of small-bodied fish species: Implications for environmental effects assessments (PO)

Keegan Hicks[1] *and Mark Servos*[1]

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Small-bodied fish species are ideal sentinel species for assessing environmental impacts because they are short lived, more abundant, and are generally thought to have reduced mobility. Although they have many advantages, there is a general lack of knowledge on the home range and mobility of most small-bodied fish species. Tracking devices to monitor the movement patterns of fish are generally limited to larger fish, thus fewer options are available to track movement of small-bodied fish. The current study used a mark-recapture technique to assess the site fidelity and movement of the rainbow darter (*Etheostoma caeruleum*), a small-bodied benthic fish species used in several recent studies assessing the impacts of municipal wastewater effluents in the Grand River watershed, southern Ontario. Rainbow darter in the urbanized area of Kitchener and Waterloo had very high rates of intersex in male fish downstream of the outfalls. However, this phenomenon was also observed in some fish collected at upstream sites in close proximity to the outfalls. This led to the hypothesis that previously exposed fish from downstream sites may be moving to the upstream reference sites. To assess site fidelity of the rainbow darter, a mark-recapture study was initiated in the upper Grand River watershed, where they were highly abundant (and collection would not affect our ongoing studies). Three riffles (50-100 m in length) along a 500 m reach, each separated by pools, were selected for the study. Each riffle was divided into 5 m by 5 m plots (108 total) and 3036 fish were tagged during July, August, and November of 2014. Fish were tagged twice, once in the abdomen using coloured elastomer (specific to each riffle) and once in the pectoral girdle with an alpha tag containing a unique code. Recaptures were assessed in August and November of 2014 and May and August of 2015 inside and outside of the original 500 m reach. A total of 565 fish were recaptured, representing an average recapture rate of 6% (30% of the recaptures retained their alpha tag). On average, 85% of recaptured fish remained in the same riffle in which they were tagged. The greatest movement was in spring (spawning period) when 30% of the fish moved to a downstream riffle, as far as 1 km. In contrast, the majority of recaptures that moved across riffles in the summer period were in the upstream direction. A high proportion of recaptures that retained their alpha tags demonstrated high site fidelity, with 70% remaining within 5 metres of their original tagging location, confirming their minimal movement. However, a small proportion of the fish may move to adjacent riffles or further during the year, possibly confounding the results (i.e., intersex) of environmental monitoring. This study successfully demonstrated a novel technique to individually track small-bodied species.

Life-stage specific differences in the sensitivity of rainbow trout to cadmium: A mechanistic study (PO)

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It is generally believed that sensitivity of fish to environmental pollutants, including metals, depends inversely on fish size. However, previous studies suggest that certain species of fish such as rainbow trout (*Oncorhynchus mykiss*) are relatively tolerant to cadmium (Cd) in the larval stage, and become comparatively more sensitive during later life-stages. To date, an understanding of the mechanistic underpinnings of life-stage specific differences in fish species, including rainbow trout, is lacking. Therefore, the main objective of this study was to investigate the mechanistic basis of the life-stage specific differences in the sensitivity of rainbow trout to Cd. We first conducted 96-hour acute toxicity assays with Cd in three different life-stages of rainbow trout: yolk sac, swim up, juvenile. Consistent with previous studies, yolk sac stage was found to be most tolerant to Cd (96-hour LC_{50}: 12.52 $\mu g \cdot L^{-1}$) relative to the other life-stages, which did not differ considerably in Cd sensitivity (96-hour LC_{50}: 1.93 - 3.0 $\mu g \cdot L^{-1}$). Subsequently, each life-stageof fish was exposed to 1 and 5 $\mu g \cdot L^{-1}$ of waterborne Cd for 40 hours, and Cd body burden and whole-body sodium (Na) and calcium (Ca) levels were evaluated. Yolk sac stage, which was most tolerant to Cd, showed no significant increase in whole-body Cd accumulation and no significant effect on whole-body Ca levels relative to the control. In contrast, a significant increase in Cd accumulation and approximately 30% reduction in whole-body Ca levels were recorded in later life-stages following exposure to Cd. Exposure to Cd did not alter the whole-body Na levels in any life-stage. Our findings demonstrated that sensitivity of rainbow trout to Cd does not necessarily depend on the body size, but rather appeared to be the result of changes in Cd accumulation and Cd-induced disruption of Ca homeostasis. Further studies are currently being conducted to determine the changes in Cd and Ca uptake kinetics across different life-stages of rainbow trout, as well as their correlation with the changes in the mRNA expression of important metal and ion transporters in the uptake epithelia (gill and skin).

Alternative Non-Vertebrate Test Methods for Evaluating Ecotoxicity

Characterization of gene expression in lake trout, northern pike and rainbow trout following exposure to EE2, fluoxetine and HBCD in vitro (PL)

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Large numbers of chemicals are discharged into aquatic ecosystems as a result of human activities. While some of these compounds have been widely studied and adverse effects on fishes have been identified, there is an increasing number of emerging contaminants, including pharmaceuticals and personal care products (PPCPs) or brominated flame retardants (BFRs), for which little or no toxicity data regarding aquatic organisms are available. Many of these emerging contaminants, such as 17α-ethynylestradiol (EE2), a potent estrogen agonist used in oral contraceptives; fluoxetine, a common antidepressant; and hexabromocyclododecane (HBCD), a widely used flame retardant, may pose significant risks to aquatic ecosystems due to their prevalence in the environment. Specifically, large quantities of these chemicals have been identified in municipal wastewater effluents (MWWEs). Widespread use of emerging contaminants has raised concerns regarding their possible risks to the environment, particularly to native species of cultural, recreational and commercial importance to Canadians, including lake trout (*Salvelinus namaycush*) and northern pike (*Esox lucius*). While extensive data are available on model laboratory species such as rainbow trout (*Oncorhynchus mykiss*), little is known of the effects of these emerging contaminants to species in northern ecosystems, and as such, approaches are needed that allow assessment of potential effects to such species. However, concerns about live animal testing, particularly of endangered or wild species, are increasing and therefore alternative testing methods such as *in vitro* tissue explant assays are needed. Following an *in vitro* tissue explant assay in which lake trout, northern pike and rainbow trout livers were exposed to serial concentrations of EE2, fluoxetine, or HBCD, transcript abundance of select genes was measured in these species and a species-specific response was characterized. With exposure to EE2, fluoxetine, or HBCD, no response was observed in expression of genes related to endocrine disruption or oxidative stress in any species tested, and therefore, these species are relatively more tolerant to these chemicals than other species previously tested.

A call for consistency: What is the most appropriate form of euthanasia for fish? (PL)

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Shell has a global animal testing policy and associated guidelines which are reviewed by an external committee and updated regularly to guarantee “best practices” for the humane use of animals. In 2014, Shell used roughly 82,000 fish for product or whole effluent toxicity testing, which is the largest category of vertebrate animal testing conducted by Shell or on its behalf. Shell's guidelines include a discussion of the euthanasia of laboratory animals. In the Shell animal testing guidelines, euthanasia means, literally, an easy or good death, and in practical terms this means that the animal should be killed causing minimal pain or distress. The guidelines reference the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia, and the Newcastle Consensus of 2006 on carbon dioxide (CO_2) euthanasia. However, no specific method of euthanasia for fish is recommended in the Shell guidelines. To remain at

the forefront of humane fish handling practices, these guidelines and the referenced documents were reviewed with the objective of identifying a method of euthanasia specifically for fish. Two common methods of euthanasia were reviewed: (1) CO_2-saturated water, and (2) buffered tricaine methanesulfonate (MS-222). Use of CO_2 with terrestrial animals can cause rapid asphyxiation and the AVMA guidelines support its use for the humane euthanasia of both terrestrial and aquatic animals, though little information actually exists supporting this position for fish. Further, other organizations such as the Canadian Council for Animal Care (CCAC) strongly advocates against the use of CO_2 for euthanasia of fish. The CCAC indicates that the addition of CO_2 to water forms carbonic acid which can alter the pH of the aquatic environment and cause undue suffering for the fish. Indeed, the AMVA guidelines warn that CO_2 might cause pain due to formation of carbonic acid on the respiratory and ocular membrane of terrestrial animals, though they do not extend this consideration to an aquatic environment. The use of the anesthetic MS-222 was also reviewed as it is currently a prominent method of euthanasia. Both the AVMA and CCAC guidelines suggest the use of an overdose of MS-222 for humane euthanasia, though recent studies suggest it might be aversive to fish. Following an extensive review of best practices and literature representing state of the science, we proposed the discontinued use of CO_2 and the use of MS-222 as the prominent method of euthanasia for Shell testing.

Applying the “3 Rs” to regulatory trout testing: A laboratory's perspective (PL)

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The acute 96-hour rainbow trout (*Oncorhynchus mykiss*) test has been used as a standard toxicity test for the permit compliance of effluents for over two decades. Within British Columbia, the provincial regulations require liquid effluents produced by industry to meet certain standards as stipulated in the *Environmental Management Act*. Independent contract laboratories who conduct these toxicity tests may use tens of thousands of juvenile rainbow trout annually to support their customers' needs. Currently, there is little pressure on the independent laboratory, from either industry or regulators, to reduce the number of fish used for permit testing. Most of the stakeholders involved continue to utilize a precautionary approach, where more fish than may be necessary are cultured, tested, and ultimately euthanized. As the importance of balancing such principles as the “3 Rs” (reduce, refine, replace) with environmental protection grows, it becomes essential to re-evaluate how fish are used by laboratories in support of permit testing. The independent laboratory is well positioned to identify many aspects of the toxicity testing process where the “3 Rs” could be applied.

Predicting the sensitivity of any oviparous vertebrate to any dioxin-like compound based on in vitro activation of the aryl hydrocarbon receptor (PL)

Jon Doering[1]*, Steve Wiseman*[1]*, Alper Alcaraz*[1]*, John Giesy*[1] *and Markus Hecker*[1]

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Adverse effects of exposure to dioxin-like compounds (DLCs) in vertebrates are primarily driven by activation of the aryl hydrocarbon receptor (AHR). Despite this single, specific, and highly conserved mechanism, differences in relative sensitivity greater than 1000-fold exist both among and within vertebrate taxa. Studies in birds have demonstrated that *in vitro* activation of AHR1 is predictive of *in vivo* sensitivity of embryos to DLCs across species of birds. However, whether this relationship holds true

for other oviparous vertebrates is unknown. Therefore, this study investigated the relationship between *in vitro* activation of AHRs in transfected COS-7 cells and *in vivo* sensitivity of embryos among fishes. Specifically, this study (1) investigated sensitivities to activation by five DLCs of AHR1s and AHR2s among nine species of fish known to differ in relative sensitivity to DLCs by almost 40-fold, and (2) characterized the relationship in fishes between *in vitro* sensitivity to activation of AHR1s and AHR2s and *in vivo* sensitivity of embryos. All AHR1s and AHR2s of fishes were activated in a concentration-dependent manner by exposure to DLCs. There was no significant linear relationship (R^2=0.24) between EC_{50} of AHR1 and LD50 of embryos. However, a highly significant positive linear relationship (R^2=0.96) was observed between EC_{50}s of AHR2s and LD_{50}s of embryos of fishes. The slope and y-intercept for this linear relationship for AHR2 of fishes is not statistically different from the slope and y-intercept for the previously determined significant linear relationship among EC_{50} of AHR1 and LD_{50} of embryos of birds to DLCs. Results of this study suggest that sensitivity to activation of AHR2, but not AHR1, mediates adverse effects of and sensitivity to DLCs among phylogenetically diverse species of fish with a comparable relationship as previously demonstrated for AHR1 of birds. This co-relationship resulted in a single equation for predicting sensitivity to any DLC across these distantly related species of oviparous vertebrates from *in vitro* sensitivity to activation of AHRs (R^2=0.88). This co-relationship between fishes and birds is suggestive that other groups of oviparous vertebrates, such as amphibians and reptiles, follow a similar co-relationship. The mechanism-based biological model developed here has the potential to guide more objective ecological risk assessment of DLCs among distantly related groups of oviparous vertebrates by use of *in vitro* methods, including species that are not easily studied, such as threatened or endangered species.

Current and emerging issues in bivalve ecotoxicology (PL)

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The lifestyle of bivalve populations makes them species at risk to anthropogenic stressors such as pollution, loss of habitats and climate changes. Indeed, mussels are sessile organisms and could live to relatively long periods in some species (up to decades if not centuries). It is anticipated that global changes are likely to have local impacts on biodiversity. In this sense, local mussel populations are directly impacted by upstream disturbances, such as urban effluent discharges and loss/modification of habitats. They are filter-feeders that trap and concentrate micro-particles in the digestive gland, which represents a major vector for contaminant exposure in mussel tissues. For these reasons, bivalves were selected as sentinel species to assess the toxicity of emerging contaminants such as nanotechnology, oil sand products, endocrine disrupters from municipal discharges, and changes in the microbiome. The cumulative effects of complex mixtures and other stressors in these times of climate change were also examined at the molecular and cellular levels, in an attempt to identify adverse outcomes pathways leading to the decline of mussel populations. Exposure to xenobiotics increases the susceptibility to temperature changes at the electron transport steps in mitochondria, which could increase energy expenses in bivalves at polluted sites under thermal fluctuations. Exposure to both municipal effluent and zinc oxide nanoparticles elicits oxidative stress, which could lead to inflammation and phagocytosis suppression. Recent studies also showed that air time survival could also be shortened in mussels exposed to nanoparticles and oil sand contaminated environments, which supports the contention that mussels are species at risk from urban activities.

Effect of lipid partitioning on predictions of acute toxicity of oil sands process-affected water to embryos of fathead minnow (Pimephales promelas) (PL)

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Oil sands process-affected water (OSPW), produced during extraction of bitumen in the surface-mining oil sands industry in Alberta, Canada, is acutely and chronically toxic to aquatic organisms. Organic compounds that are dissolved in OSPW are responsible for most toxic effects, but knowledge of specific chemicals that cause this toxicity, or their associated mechanism(s) of toxicity, is limited. Due to a lack of understanding of the toxicological effects and associated drivers of toxicity, the Alberta oil sands industry has adhered to a policy of "no release" of all process-affected materials, including OSPW. Currently, greater than 1 trillion litres of OSPW are stored in tailing ponds, all of which must be remediated and eventually returned to the surrounding environment. Due to the chemical complexity of the dissolved organic fraction of OSPW, the development of remediation methods/benchmarks and monitoring strategies has been limited. Here, we present the development of a predictive aquatic toxicity model, combining state-of-the-art mass spectrometry, measures of bioaccumulation potential for chemical species in OSPW and an understanding of mixture toxicology. Due to the complexity of the dissolved organic fraction of OSPW, traditional bottom-up mixture toxicity approaches to predict toxic potencies cannot be used. Therefore, a top-down approach to mixture toxicity predictions was developed, whereby accurate masses and not structures of chemicals are used for identification purposes by use of linear ion trap (orbitrap) ultrahigh resolution mass spectrometry. Using either solid phase micro-extraction (SPME) or solid-supported lipid membranes (SSLM), accurate masses were assigned a measured estimate of bioaccumulation. A narcosis mode of action was assumed and the target-lipid model of Di Toro et al. (2000) was adapted for toxicity predictions by use of measured bioaccumulation estimates of the individual accurate masses. Toxic potencies of mixtures were predicted assuming strict additivity. Presented results suggest that a model developed using both SPME and SSLM methods compared best with LC_{50} estimates from 96-hour acute lethality assays with embryos of fathead minnows (*Pimephales promelas*). Model predictions were within 4-fold of observed toxicity for 75% of samples and within 8.5-fold for all tested samples, well within inter-laboratory variability. In addition, the contribution of chemical classes of concern to toxicity of the mixture will be discussed. This work highlights the importance of considering differential accumulation of polar chemicals and how ultrahigh resolution mass spectrometry in combination with surrogate lipid accumulation estimates can be used to develop a model for predicting acute toxicity of fractions and samples of OSPW to early life-stages of the surrogate model fish, fathead minnow.

Shotgun proteomics of *Hexagenia* spp. exposed to surface waters and effluents from Toronto and Hamilton harbours, Ontario (PO)

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Hexagenia are an ecologically and widespread group of mayflies belonging to the order Ephemeroptera. There is growing interest in the development of a standardized test protocol using

Hexagenia spp. for use in ecotoxicological assessments. Both Hamilton and Toronto harbours were designated by the International Joint Commission as being Great Lakes Areas of Concern due to contaminated sediments from industrial activity and excess nutrients from wastewater effluents. Our goal was to characterize the effects of exposure to surface water and effluents from these two areas on the *Hexagenia* spp. proteome. Surface water samples were collected from three locations in Hamilton Harbour and two locations in Toronto Harbour. Effluents were also collected from wastewater treatment plants (WWTPs) discharging into the Harbours. Mayflies were exposed to all effluent and surface waters for 48 hours, with 10 replicates per exposure. Each animal was analyzed individually. Shotgun proteomics is a method where the proteome is first digested into peptide mixtures and then characterized by LC-MS/MS. The largest number of protein matches were found in the Diptera (flies) database, while the greatest relative number of species matches were found in the Odonata (dragonflies and damselflies) and Ephemeroptera (mayflies) databases. Significant differential expression of common proteins among all exposure and control groups suggests that there were effects on the proteome of *Hexegenia* spp. by effluents. The discussion will identify potential ways that data from this study could inform regulatory frameworks.

Unconventional Oil and Gas Development: Issues and Environmental Effects

Role of suspended solids in the toxicity of hydraulic fracturing flowback and produced wastewater (PL)

Daniel Alessi[1], Shannon Flynn[1], Yuhe He[1], Erik Folkerts[1], Yifeng Zhang[1], Dongliang Ruan[1], Jonathan Martin[1] and Greg Goss[1]

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The rapid expansion of combined horizontal drilling and hydraulic fracturing has opened previously uneconomic oil and gas deposits to development in the past decade. Many wells are fractured using slickwater, and these wells often produce large volumes of flowback and produced water (FPW) that are typically brackish and contain a suite of inorganic and organic constituents. FPW returns to the surface with a temperature representative of the geologic formation that was fractured, which in western Canada often ranges between 60°C and 120°C. Upon reaching the surface, FPW cools to ambient surface temperature, leading to the precipitation of a suite of secondary precipitates and the contemporaneous oxidation of reduced aqueous species, including ferrous to ferric iron. In this presentation, I will discuss the contribution of secondary precipitates formed in FPW collected from a well in the Duvernay Formation in Alberta, to the overall toxicity of FPW toward model aquatic organisms. FPW precipitates were thoroughly characterized using electron microscopy, energy dispersive X-ray spectroscopy, electron diffraction, and chemical digestions to understand their geochemistry. Because a range of organic constituents were found to significantly sorb to the surface of the precipitates, LC_{50} and EROD analyses were conducted on FPW with and without precipitates to understand the degree and mechanisms of toxicity due to the precipitates. Ultimately we find that secondary precipitates may comprise a large fraction of the overall toxicity of FPW, and treatment technologies to handle the precipitate fraction may prove promising in reducing the overall environmental risk posed by FPW.

Development of a guideline document for a surface and groundwater monitoring program for unconventional oil and gas exploration in the Mackenzie Valley (PL)

Bonnie Bergsma[1]

[1]Sahtu Land and Water Board[1]

In the Central Mackenzie Valley region of the Northwest Territories, interest in oil and gas exploration and long-term development is increasing. The Sahtu Land and Water Board (SLWB) must ensure that any authorization for the use of water will not substantially alter the quality, quantity, or rate of flow of waters on or flowing through or adjacent to Sahtu lands. The SLWB has issued two authorizations for hydraulic fracturing of exploratory wells. With the environmental uncertainty related to potential impacts of hydraulic fracturing activities on surface and groundwater, the Land and Water Boards of the Mackenzie Valley have developed guidelines describing minimum expectations for the preparation of a surface and groundwater monitoring program for all new water license applications for oil and gas exploration and hydraulic fracturing activities in unconventional oil and gas plays. The Guidelines set out the required design steps to implement a monitoring program: (1) set program goals and objectives; (2) develop monitoring and program design; (3) data collection and data quality; (4) data

analysis and interpretation; (5) reporting and recommendations; (6) continue monitoring. The goal of the monitoring program is to determine the baseline status or trends of the receiving environment prior to significant development (i.e., multi-well pads or permanent infrastructure construction) in order to detect any changes over time that may be the result of, or associated with, activities related to the exploration, drilling, and hydraulic fracturing. A project description, or study plan, must be developed to identify key issues and concerns about how the activity could affect the receiving environment. The nature of the activities and the contaminants of potential concern (COPC) and their pathways into the receiving environment help to identify the ecosystems/waterbodies/aquifers that are potentially at risk. Development of a Conceptual Site Model (CSM) of the possible ways the activity could affect the receiving environment helps to identify sampling locations (areas of potential concern), variables to be measured (e.g., COPC), frequency of measurement, and other key considerations for the monitoring design. A well-designed monitoring program will generate specific conclusions with an identified level of risk. The Guidelines present a three-step impact assessment monitoring approach that is initiated prior to project start-up, continues while the project is operational, and extends for a defined period post-project. The Guidelines acknowledge that the environment will differ between sites and projects and therefore allow some flexibility in design while setting a high standard of expectation for the monitoring activities. The Boards recognize that sound and sustainable water quality monitoring programs are key factors in assessing and managing potential direct, indirect, and cumulative effects to surface and groundwater quality in the Mackenzie Valley.

The ultimate mixture: The effects of extremely high salt concentrations in assessing impact of hydraulic fracturing mixtures in rainbow trout (PL)

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Hydraulic fracturing involves pumping a mixture of water, fracture proppants, and industrial fluid into deep wells at high pressures to allow for the recovery of tight gas and oil reserves. Flowback and produced wastewaters (FPW) are extremely high in salinity (up to 330,000 parts per thousand), and also contain elevated concentrations of organic and metal contaminants. From 2005 to 2013, there were more than 2500 documented flowback fluid spills in Alberta alone, and of these, 113 were large spills into flowing water, freshwater lakes or wetlands. Little data exist on the impact of these complex mixtures on native fish species. Using juvenile rainbow trout (*Oncorhynchus mykiss*), 48-hour acute toxicity tests were conducted to replicate spill effects. Fish were exposed to 7.5% of the total FPW, or salt-matched controls. Gill histology showed significant changes in lamellar width, inter-lamellar cell masses, and changes in cell type with exposure to FPW, similar to effects seen in salt-matched controls. FPW- and salt-matched control exposed fish also showed signs of oxidative stress, with decreased levels of superoxide dismutase (SOD) with 50% reduction in the highest exposure tested in the gill and liver, and increased catalase activity in the same tissues. The pattern of measured changes represents a unique signature of impact, suggesting an approach that could be used to track exposures, facilitating an understanding of spill impacts and helping to define a zone of biological influence of the spill. Overall, these data point to significant impacts of salt stress as a confounding factor in understanding the effects of FPW spills. These data will aid in the design of post-spill environmental effects monitoring and risk assessment.

Developmental toxicity of organic fractions of hydraulic fracturing flowback and produced water to early life-stages of zebrafish (Danio rerio) (PL)

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The potential environmental risk related to horizontal drilling with high-volume hydraulic fracturing (HF) has drawn a lot of public concerns. However, the toxic effects on fish embryo development posed by HF flowback and produced water (FPW) has not been well studied. FPW is a complex mixture of organic and inorganic constituents in HF fluids and deep formation water. The surface spills of FPW may cause acute and/or long-term environmental impacts on aquatic ecosystems. Previous studies indicated FPW may have endocrine-disruptive and embryotoxic effects on fish, but studies on those effects were heavily hindered due to the high mortality caused by salinity and limited exposure concentrations. In this study, the total organic fractions were extracted from sediment-free water and sediment phases of several FPW samples from various locations. Analyses of polycyclic aromatic hydrocarbon (PAH) profiles were performed for each organic fraction, demonstrating that FPW samples have complicated and highly variable organic profiles based on but not limited to PAHs profile results. Zebrafish (*Danio rerio*) embryos were exposed to various concentrations of organic fractions for 120 hours. Mortality (LC_{50}), success of hatching, survival, and incidences of spinal malformation were examined. A scale of degree of spinal malformation was designed and applied to calculate the cumulative curvature score (CCS) for each exposure group. Compared to the previous studies using the dilutions of original FPW samples, the organic fractions of FPWs have much higher LC_{50} values, proving the major lethal toxicity is dominated by high salinity in original FPW samples. Generally, exposure to organic fractions of FPW significantly increased the incidences of morphological deformities in zebrafish embryo development, resulting in higher CCS. Expression of a series of genes related to biotransformation, oxidative stress and endocrine disruption was also found to be altered in exposure groups through quantitative real-time polymerase chain reaction. The results provide novel information on the developmental toxicity of organic contents in HF-FPWs, and suggest that the organic contaminants released from FPW spills may have potential long-term environmental effects on teleost early development.

Respirometry and swim performance alterations in zebrafish (*Danio rerio*) acutely exposed to hydraulic fracturing flowback and produced water (PL)

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Horizontal hydraulic fracturing is an emerging practice in North America to extract oil and gas reserves. To date, little toxicological information is supplied for both the technologies and chemicals used during the fracturing process, and for the surface returned by-product fracturing flowback and produced water (FPW). Volumes of water used per well can often exceed 1 million m^3, resulting in potentially devastating environmental effects when spills of FPW occur. Although salinity dominates much of the toxicity observed, other inorganic (e.g., metals, radionuclides) and organic (e.g., polyaromatic hydrocarbons, benzenes) molecules found in FPW can induce toxicological responses in exposed organisms. The current study uses zebrafish (*Danio rerio*) as a model organism to determine if acute exposures to FPW induce developmental changes in organismal oxygen uptake. To determine if exposure to dilute FPW resulted in changes in oxygen consumption, zebrafish embryos were acutely exposed to 2.5% and 5% FPW and oxygen consumption was measured. Transcriptional analyses of

specific hypoxia, stress inducible, and cardiovascular genes were performed and paired with embryo metabolic rates to determine if FPW exposure affected immediate embryo oxygen consumption, and if FPW-induced respirometry changes persisted after exposure was terminated. Furthermore, developmental toxicity assays were employed to determine if acute FPW exposure changed developmental deformity (such as pericardial edema and yolk sac edema) occurrences in 120-hour post-fertilized larvae. Finally, juvenile zebrafish swim performance experiments were performed to determine if acute FPW exposure affected later stage fish fitness. This is one of the first studies to use respirometry as a metric for measuring FPW induced toxicity. Our results aim to validate respirometry as a potential toxicological biomarker for identifying biological zones of impact when FPW spills occur, and provide regulatory agencies information to help shape future FPW spill/leak remediation protocols.

Isolation and characterization of the primary toxic components in oil sands process-affected water (PL)

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Previous research linked the primary toxicant in various sources of oil sands process-affected water (OSPW) to the organic acid fraction (naphthenic acids), but did not have access to the high-resolution mass spectrometry techniques that are now available to measure and characterize the organic acid component of OSPW. More recent studies have used these techniques to implicate O_2 (i.e., classical) naphthenic acid species as the primary cause of toxicity, but because of methodological and statistical limitations, these studies were unable to demonstrate quantitative dose-response relationships. This study characterized the primary toxic component(s) in OSPW using the Toxicity Identification Evaluation (TIE) approach. Phase 1 TIE treatments were used to identify the general characteristics of constituents responsible for toxicity using acute 96-hour rainbow trout (*Oncorhynchus mykiss*) exposures. Toxicity was primarily associated with the organic fraction, and toxic components were further separated using solvent-gradient fractionation. Naphthenic acid fraction compounds in the toxic and non-toxic fractions were determined using orbitrap mass spectrometry in negative ion mode, and the data were used to conduct a principal components analysis (PCA). The resultant PCA axis loadings were then used to conduct regression analyses between various classes of naphthenic acids and toxicity, with only the O_2 class (classical naphthenic acids) demonstrating a significant relationship. To our knowledge, this is the first time that a quantitative relationship has been demonstrated between classical naphthenic acids and acute toxicity. Of note, this investigation also found evidence of a secondary toxicant, and additional work is being undertaken to determine its identity. The results of this research increase our understanding of the link between naphthenic acids and OSPW toxicity, and aids in identifying other toxic constituents in OSPW. Moreover, these data will help develop treatment goals and targets for removal of OSPW toxicity in future mine closure landscapes and water return scenarios.

Population impacts in white sucker (*Catostomus commersonii*) exposed to oil sands-derived contaminants in the Athabasca River (PL)

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The rapid development of the Athabasca oil sands in Alberta, Canada, has raised concerns about the ecological impacts on downstream aquatic environments, particularly the Athabasca River. Population, physiological and biochemical responses in white sucker (*Catostomus commersonii*) collected downstream of Athabasca oil sands developments were compared with those at Calling Lake, a location upstream of oil sands activities. These analyses were paired with an evaluation of waterborne naphthenic acids in the river and indicators of exposure to naphthenic acids, polycyclic aromatic hydrocarbons and metals in white sucker tissue. Levels of naphthenic acids were elevated in tributaries adjacent to oil sands mining activities but were lower in the mainstem of the river. Tributary naphthenic acid profiles were more similar to oil sands aged tailings water than were the mainstem water samples, suggesting some influence of tailings in the tributaries. White sucker showed higher energy storage in the Athabasca River as indicated by significantly higher condition and liver size in both male and female fish. White sucker were not investing that energy into reproductive effort as measured by gonad size in the two sexes and fecundity in females, which were significantly reduced relative to the reference location. Despite the differences in gonad development, there were no significant differences in white sucker sex steroid hormone concentrations between the two locations. Athabasca River white sucker showed increased exposure to polycyclic aromatic hydrocarbons as indicated by hepatic CYP1A activity and fluorescent bile metabolites. Naphthenic acids measured in bile also indicated exposure to low concentrations of these compounds in the Athabasca River. Some priority metals (e.g., cadmium, copper, nickel, and selenium) were also elevated in white sucker liver tissue when compared to the reference location. Under the current regime of oil sands mining in the region, compounds such as polycyclic aromatic hydrocarbons and metals derived from atmospheric deposition currently present a greater risk to fish populations than naphthenic acids derived from natural sources or tailings seepage.

Investigating the mechanism of toxicity of vanadium to *Daphnia* spp. (PO)

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Insufficient data are available to understand how common water quality variables representative of Alberta's oil sands region can affect toxicity of vanadium (V) to aquatic organisms and how this effect could be explained through an understanding of the toxic mechanisms of action of V. Ongoing investigations proposed the use of petroleum coke to reduce organic toxicants through sorption present in oil sands process-affected water (OSPW). However, vanadium is released from petroleum coke to "coke-treated" OSPW, increasing the aqueous V concentration from negligible to up to 7 $mg \cdot L^{-1}$. Results to date indicate that some water chemistry variables modify V toxicity to aquatic organisms. For instance, when alkalinity increased from 100 to 600 $mg \cdot L^{-1}$ as $CaCO_3$, the toxicity of V to *Daphnia pulex* decreased. Also, when sulfate concentrations were raised from 30 to 300 $mg \cdot L^{-1}$, the LC_{50} to *D. pulex* rose from 0.95 to 1.31 $mg \cdot L^{-1}$. However, when the concentration of sodium was higher than 400 $mg \cdot L^{-1}$, the toxicity of V to *D. pulex* doubled. Sulfate and bicarbonate have similar chemical structure to vanadate

(the most abundant species at the tested pH), so these contrasting trends could indicate that the mechanism of V uptake and toxicity in *Daphnia* could be through anion carriers, and that V may be affecting the internal sodium ion balance, potentially causing a respiratory disruption within the organism. Other research has suggested that V inhibits the sodium-potassium pump, while separate publications suggest that V elicits its toxicity through generation of reactive oxygen species. Thus, there is still uncertainty as to what the actual mechanism of toxicity is. Here, a mechanistic investigation of V toxicity using *Daphnia magna* is described. Initial results indicate that there is no significant effect of V on whole-body sodium concentration or on sodium influx in *D. magna*, suggesting that the inhibition of the sodium-potassium pump is not the most probable mechanism of toxicity. Further research will focus on lipid peroxidation and effects of V on superoxide dismutase (SOD) and catalase (CAT) activity to investigate if oxidative stress is the predominant mechanism of action.

Current-Use Pesticides: Fate and Effects in Canadian Ecosystems

Pesticides in Alberta's groundwater: Results of a three-year study (PL)

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This project aimed to determine the occurrence of agricultural pesticides in groundwater of southern and central Alberta. To this end, groundwater samples (440) were collected from water table wells and piezometers over a three year period (2013-2016). In addition, shallow groundwater (214) and soil (122) samples were collected from experimental plots at the Lethbridge Research and Development Centre of Agriculture and Agri-Food Canada. All samples were extracted and analyzed for the presence of 106 (2013, 2014) or 146 (2015) pesticides. In total, 23 different pesticides were detected in groundwater collected from agricultural land in central and southern Alberta, while 13 pesticides were detected in shallow groundwater collected from experimental plots. Moreover, 35 different pesticides were detected in soil samples collected from the experimental plots. Pesticides detected included herbicides, insecticides and fungicides. Pesticides detected most frequently at all locations were herbicides, although differences in the spatial and temporal distribution of pesticides were observed. Overall pesticide detection frequency and concentration in groundwater of Alberta are less than those found in surface waters. In addition, fewer pesticides were detected in groundwater compared to surface waters. Concentrations measured for all pesticides were well below water quality guidelines, as well as maximum acceptable concentrations (MAC) for drinking water.

Persistence of Garlon™ XRT (triclopyr) and Arsenal® PowerLine (imazapyr) in soils along transmission right-of-ways in the Yukon (PL)

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Currently, brushing and mowing are the only vegetation management techniques used along transmission right-of-ways in the Yukon territory. When mowed, dominant woody species such as *Populus tremuloides, Populus balsamifera* and *Salix* spp. grow rapidly. The prolific growth shortens management cycles, increasing associated costs. Different vegetation management options were reviewed resulting in the implementation of a pilot study addressing the effectiveness, persistence and toxicity of different herbicide application techniques. Specifically, this research aims to address the persistence of Garlon™ XRT (triclopyr) and Arsenal® PowerLine (imazapyr) in soils along transmission right-of-ways in the Yukon. Three different application techniques (backpack spray, cut stump and point injection) were assessed in 6 m x 6 m treatment plots at five sites representing the main ecoregions where right-of-ways occur in the Yukon. Soils from one site were collected as soon as herbicides had dried and at 1, 3, 7, 14, 21, 30, 60 and 365 days after treatment to develop a detailed dissipation curve. Soil samples from the remaining four sites were collected at 1, 30 and 365 days after treatment. Herbicide residues from select samples are being analyzed using high performance liquid chromatography coupled with mass spectrophotometry. The dissipation curve for triclopyr from the backpack spray treatment indicates that the half-life occurs within 7 days of application, with residues at all sites persisting for longer than 30 days. Backpack spray samples from 365 days after treatment are currently being analyzed to further assess dissipation. In the cut stump and point injection treatments,

residues are present in the soil at 30 days after treatment, but are reduced by greater than 80% in the point injection treatments and are below analytical detection limits in the cut stump treatments at 365 days after treatment. Imazapyr samples are currently being analyzed. Further research is being conducted to determine the impact these residual concentrations may have on the soil ecological community in the Yukon.

Refined hazard assessment of the polyoxyethylene amine surfactant in Roundup®: The importance of sediment in aquatic toxicology (PL)

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The actual hazard/risk posed by MON 0818, the polyoxyethylene amine (POEA) surfactant present in the original formulations of glyphosate (e.g., Roundup® Original), has traditionally been hard to assess, mainly due to lack of exposure data. Here, we present a refined hazard assessment of MON 0818 based on the re-evaluation of the existing toxicity data, supplemented by water-only toxicity data for an additional 15 species, tested with standard methodologies and confirmation of exposure concentrations. Species Sensitivity Distributions were compared against a series of scenarios showing moderate levels of hazard (43.1% of the species exposed at or above EC_{50} levels) for a chosen worst-case scenario: unintentional direct over-spray of a 15 cm-deep body of water with the maximum label application rate for the studied formulations (Roundup® Original, Vision®; 12-L formulation·ha^{-1}, equivalent to 4.27 kg acid equivalent (a.e.) per hectare (ha)). The hazard decreased to impairment of 20.9% of species under the maximum application rate for more typical uses (6-L formulation·ha^{-1}, 2.14 kg a.e.·ha^{-1}), and down to a 6.9% for a more frequently employed application rate (2.5-L formulation·ha-1, 0.89 kg a.e.·ha^{-1}). Finally, the percentage (3.8%) was less than the hazardous concentration for 5% of the species (HC_5) based on concentrations of MON 0818 calculated from maximum measured concentrations of glyphosate in the environment. In addition, we recently showed that MON 0818 presents a high affinity for sediment and that, in its presence, the water column half-life of the surfactant can be very short (generally <10 hours, and commonly <5 hours). In order to further explore the role that sediment plays in the exposure of aquatic organisms to MON 0818 we also present the results of a series of tests following novel approaches such as water column toxicity tests in the presence of sediment and the evaluation of pulse-recovery approaches to mimic water column exposures for compounds with short water-column half-life. When evaluated in this manner, no acute (24-hour) mortality was observed for three of the five species tested in the presence of clean sediment up to 10 mg·L^{-1} MON 0818 initial water concentration. The EC_{50}s for the other two species were significantly greater than for parallel water-only tests. Our results indicate that quick dissipation of MON 0818 in the presence of sediment can reduce the effects on exposed organisms, and that full recovery from 24-hour exposures to concentrations of MON 0818 equal to, or greater than, those expected in the environment is possible. These results highlight the inappropriateness of simple screening-level approaches for the assessment of the risk posed by compounds for which the expected exposure regimes may deviate from that more typically used in traditional standard acute laboratory tests.

Pesticides in effluents, tributaries and mainstem rivers in Alberta's South Saskatchewan River Basin: A synoptic survey (PL)

Natalie Kromrey[1], Claudia Sheedy [2] and Denise Nilsson [2]

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A synoptic (time of travel) study was performed beginning on August 12, 2014, to determine if pesticide concentrations, number of pesticides detected and frequency of detection were highest in mainstems, tributaries or effluents. Three parcels of water were theoretically followed for eleven days using hydrological time of travel calculations from their respective headwaters in the Red Deer, Bow, and Oldman Rivers to their eventual convergence in the Province of Saskatchewan. Water quality samples were taken at 68 mainstem river stations, 63 tributary stations and 24 effluent sources. They were sampled for over 200 parameters including physical and biological parameters, nutrients, total and dissolved metals, bacteria and a suite of 106 pesticides. 2,4-D, Dicamba, Mecoprop (MCPP) and MCPA had the highest number of detections as well as the highest concentrations. The frequency of detection and number of different pesticide detections were highest (83.3%, 17) in wastewater treatment effluent (6 industrial, 18 municipal stations), which were greater than those at tributary stations, which in turn were greater than those at mainstem stations. Pesticide detections in effluent sources exceeded expectations, prompting a winter resampling of a representative subset of effluents (2 industrial, 6 municipal; encompassing all four basins). The frequency of pesticide detection of the winter effluent samples was comparable to that of the summer, while the number of different pesticide detections and concentrations was lower. Pesticide monitoring is not required by Alberta Environment and Parks at all of the facilities sampled. In light of the results, to better determine the contribution to the environment, consideration could be given to the merits of incorporating a pesticide monitoring requirement into all operating approvals for effluents that discharge to a receiving waterbody. Moreover, consideration could be given to the confounding factor pesticides may play in ecotoxicity experiments involving wastewater effluents or receiving waters.

Modelling the cumulative toxicity of neonicotinoid insecticide mixtures using MIXTOX analysis (PL)

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Neonicotinoids are a popular class of systemic insecticidal treatment, commonly applied as a seed treatment to protect young crops against biting-sucking insects. Extensive use of these seed treatments (e.g., imidacloprid, clothianidin, and thiamethoxam) in Canadian Prairie agricultural regions has resulted in the detection of neonicotinoid mixtures in ecologically important wetlands surrounding these arable areas. However, the impact of these mixtures on local insect communities is poorly understood. This research aims to address that knowledge gap by characterizing the cumulative toxicity of binary and tertiary mixtures of select neonicotinoids (imidacloprid (IMI), clothianidin (CLO), and thiamethoxam (TMX)) to the sensitive aquatic insect *Chironomus dilutus* under acute (96-hour) exposure scenarios. Single-compound toxicity bioassays yielded toxicity threshold values (LC_{50}) of 4.63 (3.96 - 5.41), 5.93 (5.29 - 6.65), and 55.34 (43.98 - 69.64) for IMI, CLO, and TMX, respectively. These values were used to develop parametric models, which were statistically compared to the toxicity of binary and ternary mixtures from similar laboratory studies using the MIXTOX approach. CLO-TMX mixtures demonstrated concentration-additive synergistic toxicity. IMI-CLO mixtures demonstrated response-additive synergistic

toxicity. IMI-TMX mixtures demonstrated response-additive dose-ratio dependent synergism, with toxicity shifting from antagonism to synergism as the relative concentration of TMX was increased. IMI-CLO-TMX mixtures demonstrated response-additive synergistic toxicity. Results obtained indicate that under acute exposure scenarios, the toxicity of these neonicotinoid mixtures to sensitive aquatic insects cannot be adequately predicted by direct addition of single compound concentrations. These results will be compared to those garnered in concurrent chronic (28-day) mixture studies, allowing for an assessment of whether these insecticide mixtures display similar deviations from concentration addition under more environmentally realistic exposure paradigms, and what risk this may pose to the aquatic environment.

Effects of neonicotinoid pesticides on non-target invertebrates in the terrestrial environment (PL)

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Neonicotinoids are being intensively used on a variety of crops and have the potential to continuously expose non-target organisms. For terrestrial invertebrates, exposure through contact with contaminated soil, water or plants can result in lethal and sublethal effects, with most information gathered from honey bee (*Apis mellifera*) studies. However, it is important to assess a variety of non-target taxonomic groups to fully understand the scale and magnitude of effects. We examined the direct effects of neonicotinoids on an herbivorous invertebrate, using the generalist grasshopper *Melanoplus sanguinipes* as a representative non-target species occupying field margin habitats. We also conducted soil toxicity assays using two invertebrate species, the earthworm *Eisenia andrei* and the springtail *Folsomia candida* to assess for the effects on survival and reproduction; very few studies have assessed the risk of neonicotinoids to soil-dwelling species, despite growing evidence of soil persistence. We orally exposed adult grasshoppers to a gradient of concentrations of the formulated neonicotinoid product containing clothianidin. We monitored consumption, biomass gain and sublethal effects (e.g., lethargy and mobility) over a 4-day exposure period. For the soil invertebrate assays, we exposed adult organisms to a formulated neonicotinoid product (active ingredient thiamethoxam) in a natural sandy loam soil. Test endpoints included adult survival after 28 days, and juvenile production after 28 (*F. candida*) or 56 (*E. andrei*) days of exposure. Grasshopper and earthworm tissues were also analyzed to assess the product's bioaccumulation potential. Preliminary results indicate that grasshoppers were affected by concentrations less than the recommended application rate and sublethal effects were detected for *F. candida* at <0.5 $mg \cdot kg^{-1}$ and for *E. andrei* at <10 $mg \cdot kg^{-1}$. This project provides base-line toxicity and bioaccumulation data on the effects of neonicotinoids to non-target invertebrates that are important for terrestrial food webs and soil health.

Do neonicotinoid insecticides pose a hazard to the early life-stages of freshwater mollusks? (PL)

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Neonicotinoid insecticides can be transported from agricultural fields, where they are used as foliar sprays or seed treatments, to surface waters by surface or sub-surface runoff. Few studies have investigated the toxicity of neonicotinoid or the related butenolide insecticides to freshwater mollusk species. The current study examined the effect of neonicotinoid exposure to the early life-stages of the ramshorn snail (*Planorbella pilsbryi*) and the wavy-rayed lampmussel (*Lampsilis fasciola*). Juvenile *P. pilsbryi* were exposed to imidacloprid, clothianidin, or thiamethoxam for 7 or 28 days and mortality, growth, and biomass production were measured. The viability of larval (glochidia) *L. fasciola* was monitored during a 48-hour exposure to six neonicotinoids (imidacloprid, thiamethoxam, clothianidin, acetamiprid, thiacloprid, dinotefuran), or a butenolide (flupyradifurone). The 7-day LC_{50}s of *P. pilsbryi* for imidacloprid, clothianidin, and thiamethoxam were ≥ 4000 $\mu g \cdot L^{-1}$ and the 28-day LC_{50}s were ≥ 182 $\mu g \cdot L^{-1}$. Growth and biomass production were considerably more sensitive endpoints than mortality with EC_{50}s ranging from 33.2 to 122.0 $\mu g \cdot L^{-1}$. The LC_{50}s for the viability of glochidia were ≥ 456 $\mu g \cdot L^{-1}$ for all seven insecticides tested. Our data indicate that neonicotinoid and butenolide insecticides pose less of a hazard with respect to mortality of the two species of mollusk compared to the potential hazard to aquatic insects.

The effects of the herbicide diquat on aquatic biota (PO)

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Invasive aquatic plant species are a significant threat to the health of aquatic ecosystems, and demand for chemical control options is likely to increase as invasive species spread and reach nuisance levels. Currently, data on the effects of chronic, ecologically relevant concentrations of current-use aquatic herbicides on non-target biota are lacking. The objective of our study was to assess the effects of the aquatic herbicide diquat on target and non-target biota using outdoor 300-L mesocosms to simulate natural systems. The experiment consisted of a control and five treatments reflecting environmentally relevant concentrations of diquat, each with five replicates. Biota included: (1) native and invasive macrophytes collected from nearby waterbodies (*Elodea canadensis, Ceratophyllum demersum, Hydrocharis morsus-ranae* and *Myriophyllum spicatum*), (2) natural communities of phytoplankton and periphyton, (3) caged amphipods (*Hyalella azteca*), and (4) northern leopard frog tadpoles (*Lithobates pipiens*). The effects of diquat on biota important to Canadian ecosystems will be discussed. Overall, results from this study will assess the potential risks versus benefits diquat poses to the aquatic environment.

Sublethal effects of the neonicotinoids imidacloprid and thiamethoxam on developing sockeye salmon (*Oncorhynchus nerka*) (PO)

Paula Duarte-Guterman[1], Sean Engelking[1], Debra Reeves[1], Cody Antos[1], Chris Kennedy[1] and Vicki Marlatt[1]

[1]*Simon Fraser University*

Neonicotinoids are a class of widely used insecticides that have been detected in ng–µg·L^{-1} concentrations in surface waters worldwide near agricultural waterways, but few studies have examined the potential sublethal effects of these pesticides on aquatic vertebrates. In particular, the health effects of chronic, low-level neonicotinoid exposure on anadromous salmonids within the Fraser River Basin in British Columbia, Canada, are poorly understood. This research examined the effects of the neonicotinoid insecticides, imidacloprid and thiamethoxam, following either a single pulse or chronic exposure, on critical early life-stages of a wild salmon species, sockeye salmon (*Oncorhynchus nerka*). In these experiments, four concentrations of the neonicotinoids (0.15, 1.5, 15 and 150 µg·L^{-1}) plus a water control were tested in a single pulse exposure experiment during the fertilization process (imidacloprid only) and in a chronic exposure experiment that was initiated immediately post-fertilization (imidacloprid and thiamethoxam). All experiments continued through to the swim-up fry developmental stage. Three unique offspring sets (crosses) in the imidacloprid exposure and two unique crosses in the thiamethoxam exposure were tested within each experiment, and all treatments were conducted in duplicate. Neither of the neonicotinoids at the concentrations tested had any effects on survival rates during these studies. Analyses are ongoing; however, to date no effects are evident on body length or weights in either the pulse or chronic continuous pesticide exposures, but significant differences in morphometrics between genetic crosses are apparent. In addition, preliminary gene expression analyses in fish exposed to imidacloprid revealed cross- and treatment-specific effects on oxidative stress and detoxification biomarkers. Work is underway to explore the differences between genetic crosses and neonicotinoid-induced molecular responses (hepatic endocrine and immune system transcript abundance) and hormone concentrations to further evaluate the sublethal effects of these pesticides in early life-stage sockeye salmon. Supported by Fisheries and Oceans Canada National Contaminants Advisory Group.

The effects of anti-sea lice therapeutants on sensitive life-stages of non-target species in combination with different stressors (PO)

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There is specific concern regarding the use of the anti-sea lice therapeutants Salmosan®, SLICE®, and Paramove 50® in the aquaculture industry and their toxicity to Pacific coast region organisms; it is unclear if current data using Atlantic species can be considered a surrogate for species indigenous to the west coast of Canada. Equally unknown are the potential lethal and sublethal effects that may occur in sensitive species at aquaculture sites where other stressors of a physical nature (e.g., hypoxia, temperature fluctuation) exist. Several shrimp species were tested for the acute lethal effects of each chemical: the reference species mysid shrimp (*Mysidopsis bahia* East coast species), and several species of Pacific shrimp including the coonstripe (*Pandalus hypsinotus*), dock (*Pandalus danae*) and pink shrimp (*Pandalus jordani*), spot prawn (*Pandalus platyceros*), ghost shrimp (*Neotrypaea* spp.), and an unidentified sand shrimp. Shrimp were exposed to 5 concentrations of either azamethiphos, hydrogen

peroxide or emamectin benzoate in 40-L glass aquaria for up to 96 hours. Preliminary analysis of these data indicate that Pacific coast shrimp are equally as sensitive to all three chemicals as their East Coast counterparts. Initial experiments involved exposing spot prawn to water containing emamectin benzoate, azamethiphos or hydrogen peroxide and measurement of oxygen consumption (stress indicator). Chemical exposure of all three compounds resulted in concentration-dependent increases in oxygen consumption. Spot prawns were not exposed to aquaculture chemicals in a second set of experiments and were acclimated in a shuttle box apparatus, in a choice/avoidance assay. Hydrogen peroxide, emamectin benzoate, and azamethiphos at several concentrations showed conflicting results. Prawns were actively attracted and actively avoided all chemicals at low concentrations. In another set of experiments, it was necessary to determine the range of oxygen, temperature, and salinity tolerances in adult *Pandalus* spp. and to determine ranges for each of these "stressors" to accompany aquaculture chemical exposures. Tolerable ranges have been determined that are currently being utilized for acute toxicity and sublethal toxicity experiments. The data obtained from this research are required to ensure the proper and safe use, and appropriate regulation of these aquaculture chemicals in Canada.

Investigation on the occurrence of neonicotinoid insecticides in Ontario wastewater treatment plants (PO)

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Neonicotinoid insecticides are nicotine-based systemic compounds that are widely used in agriculture, either in field applications or as seed treatment, for the control of insect pests on vegetables, cereals, potatoes, corn, berries, tree fruits, and in turf and ornamental crops. Other uses include pet flea and tick products, treatment of structural areas (e.g., livestock production facilities), injection into trees, as well as use in greenhouses and nurseries. Studies have linked neonicotinoid insecticides with adverse impacts on non-target organisms such as pollinators (e.g., honey bees). In order to protect non-target organisms, the Government of Ontario introduced new regulatory requirements for the sale and use of neonicotinoid-treated seeds, to reduce the number of acres planted with neonicotinoid-treated corn and soybean seed by 80% by 2017. While attention has largely focused on potential risk to beneficial terrestrial invertebrate insects, the detection of neonicotinoids in aquatic habitats indicates the potential for risk to aquatic organisms as well. A recent study published by Arizona State University showed detections of imidacloprid and other neonicotinoids in the influent and effluent of 13 conventional wastewater treatment plants. In this study, the occurrence of neonicotinoid insecticides in Ontario wastewater treatment plants is reported, with a discussion on the implications this may have to receiving water non-target aquatic organisms.

Trends in Behavioural Toxicology

Soil invertebrates' avoidance of hydraulic lubricating oil (PL)

Amy Gainer[1] *and Steven Siciliano*[1]

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Avoidance tests are a rapid toxicity test type in soil toxicology to assess the influence of contaminants on behaviours of organisms. Behavioural studies are currently lacking in ecotoxicology risk assessment and can easily be incorporated into the common risk assessment tool, the species sensitivity distribution (SSD) curve. Soil invertebrates with chemosensing abilities avoid non-volatile, volatile and semi-volatile contaminants. Semi-volatile contaminants, like petroleum hydrocarbons, interfere with chemosensing and alter the distribution and behaviour of soil invertebrate populations. Soil guidelines should be developed with consideration of more ecologically relevant endpoints than just reproduction and mortality as it is important to maintain ecological functioning of soil invertebrate communities following contamination events. The effective concentration (EC) values for avoidance tests for numerous soil invertebrates were compared to the EC values for reproduction. Soil remediation guidelines derived from a SSD were determined with and without inclusion of avoidance tests to assess how these tests influence the resulting guidelines.

The effects of oil sands processed-affected water on *Daphnia magna* (PL)

Dylan Steinkey[1]*, Ebrahim Lari*[1] *and Greg Pyle*[1]

[1]*University of Lethbridge*

Present production in the Alberta oil sands has raised concerns about its prospective influences on the surrounding environment. This study has demonstrated toxicity from oil sands process-affected water (OSPW) to *Daphnia magna* exposed to OSPW as well as its dissolved component (DC) and suspended particulate matter (SPM). All components demonstrate a reduced feeding rate compared to unexposed *D. magna*. This study also examined different mechanisms of action to explain the observed feeding rates seen in each group. Digestive enzymes trypsin and amylase were largely unaffected by the individual components; however, there was a significant decrease in trypsin activity when exposed to total OSPW (as normally found in tailings ponds). Neither mandible rolling nor post-abdominal rejections, two feeding behaviours, showed change after any treatment. Thoracic limb movements, which provide water flow toward the feeding groove, were reduced in all SPM and total OSPW treatments but not in the DC treatment. Peristaltic movements were reduced, resulting in a reduction of digestion time in all DC treatments. Energy dispersive x-ray analysis detected both aluminum and silicon in SPM treatments. All exposures showed an increase in the number of intact algae cells after excretion, demonstrating that the reduction in feeding rates is partly caused by improper digestion.

Immediate interaction of oil sands process-affected water with the olfactory system of fish (PL)

Ebrahim Lari[1] *and Greg Pyle*[1]

[1]*University of Lethbridge*

Determining the potential effects of oil sands process-affected water (OSPW), the main by-product of the oil sands surface mining process, on aquatic ecosystems is of main concern to oil sands companies and legislators concerned about the reclamation of mining sites. In the present study, the first interaction of OSPW with the chemosensory system of rainbow trout (*Oncorhynchus mykiss*) was investigated. The aim of the first part of the study was to determine whether or not the olfactory system of fish detect OSPW. The aim of the second part of the study was to investigate if the olfactory fatigue resulted from continuous exposure to OSPW thereby reducing the acuity of olfactory system of rainbow trout. Olfactory acuity and its response to chemical cues was tested using electro-olfactography (EOG). In order to find out whether or not rainbow trout can detect OSPW using olfaction, fish were tested for their avoidance behaviour in a choice maze and EOG response to 1 and 10% OSPW. To investigate the immediate effect of OSPW on the olfactory acuity of rainbow trout, fish were tested for their attraction and avoidance behaviour in a choice maze and EOG response to a food (L-alanine) and an alarm (taurocholic acid) cue in presence and absence of OSPW. Rainbow trout ware able to detect OSPW at concentrations as low as 1%. The presence of OSPW at 1% and higher also reduced olfactory acuity. The results of the present study suggest that fish can detect and probably avoid OSPW if possible. If the fish cannot detect or avoid OSPW for any reason, its ability to detect natural chemosensory cues could be impaired immediately after being exposed to OSPW.

The antidepressant venlafaxine impacts development and behaviour in larval fathead minnow (PL)

William A. Thompson[1] *and Mathilakath Vijayan*[1]

[1]*University of Calgary*

Antidepressants prescriptions have been increasing worldwide, resulting in higher levels of these neuroactive compounds in municipal wastewater effluent (MWWE). These compounds are biologically active in humans, but the impacts on non-target organisms are far from clear. In this study we used the fathead minnow (*Pimephales promelas*), a species native to Alberta, as a model to understand the impact of the antidepressant venlafaxine (Effexor) on development and behaviour. Venlafaxine is a selective serotonin and norepinephrine reuptake inhibitor prescribed for mood alteration in humans. It commonly appears in MWWE at concentrations upwards of 0.5µg·L^{-1} worldwide. We hypothesized that venlafaxine would alter the behavioural response and the normal development of the fathead minnow. Fertilized fathead minnow eggs were exposed to environmental concentrations of venlafaxine (0, 0.1, 0.5, 1, 2, and 4 µg·L^{-1}) for 7 days and key developmental markers, including survival, hatching success, growth and behaviour, were assessed. Venlafaxine exposure did not affect survival, but altered the time of hatch and growth in the exposed embryos. The behavioural end-points included thigmotaxis, commonly used in zebrafish to assess the anxiety response, and swimming performance. Overall, venlafaxine affects key stages of fathead minnow development, while the behavioural models used in this study may be useful as a high-throughput screening tool to assess the impacts of environmental levels of antidepressants on fish performance. This study was supported by the Natural Sciences and

Engineering Research Council of Canada Discovery and Strategic Grants (to Vijayan) to MMV and an Eyes High Doctoral Scholarship (to Thompson).

Modelling zebrafish embryo photomotor responses to test for the effects of contaminants and their mixtures (PL)

Patrick Gauthier[1] and Mathilakath Vijayan[1]

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Animal behaviours in response to a stressor are complex and adaptive strategies to maximize survival and fitness. While animals typically have a relatively stable activity profile under non-stressful conditions, the sudden occurrence of a predator or environmental change drastically alters this profile. Dealing with such complicated activity data is often handled by averaging activity over an interval of time (e.g., total activity pre- and post-stimulus). This avoids substantial statistical headache, but greatly simplifies animal behaviour as the change in activity is typically dynamic and non-linear. A researcher may be interested in knowing the complete profile of how an animal responds to an environmental stimulus and might ask: does activity change and if so, how rapid is the change in activity, at what point in time does that change in activity reach a maxima, and what is that maxima? Moreover, ecotoxicologists will be interested in how the presence of a contaminant influences these metrics, as they provide highly ecologically relevant information regarding animal function, performance, and survival. Thus, finding appropriate models that encapsulate these metrics will prove exceptionally useful and help propel the use of animal behaviour as an ecotoxicological endpoint. Because environmental exposure occurs as a mixture of contaminants and the effects of a mixture can diminish or enhance the overall toxicity of the contaminants that are present, understanding these outcomes is critical for predicting potential adverse effects of mixtures in the environment. Unfortunately, there is no current consensus on appropriate statistical methods for predicting mixture toxicity from a regulatory standpoint, and no clearly outlined modelling approaches for simultaneously analysing time-series behavioural data for: (1) the effect of contaminants on behaviour, and (2) the additive effect of those contaminants on behaviour. We present a novel statistical approach with the zebrafish (*Danio rerio*) embryo photomotor response (PMR) assay. We apply non-linear mixed effects (nlme) modelling to test for the effects of β-adrenergic compounds and their mixtures on the complete time-series of the PMR of zebrafish embryos. We found the nlme model performed excellently in describing the PMR time-series data and revealed that exposure to the β-agonist isoproterenol and the β-antagonist atenolol stimulated and supressed the embryo PMR, respectively. However, exposure to the β-agonist and β-antagonist mixture produced non-additive effects on the zebrafish embryo PMR. The successful application of the nlme modelling on behavioural data, particularly in the context of a mixture, has potential to improve the use of animal behaviour in ecotoxicological research.

A comparison of exposure to diluted bitumen and conventional crudes on visual learning of *Danio rerio* larvae (PL)

Clara Philibert[1], Danielle Philibert[1] and Keith Tierney[1]

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In North America, it is predicted that current pipelines will not be able to handle the demand for oil and natural gas, creating the need for expansion. With this growth it is likely that the incidences of

pipeline spills will also grow. In 2012 there were 140 incidents of pipeline spills in Canada and this number is projected to increase as new pipelines are built. With continued drilling and oil sands exploration there are both conventional and unconventional crudes that are marketed throughout North America and overseas. Two common conventional crudes that are currently being shipped in Alberta are mixed sweet blend (MSB) and medium sour composite (MSC). Bitumen extracted from the oil sands is unconventional crude and is mixed with natural gas condensates, creating diluted bitumen (dilbit) which then can be shipped via pipeline. It is critical that we understand the effects of these three common pipeline oils on aquatic organisms so that we can better assess the risks associated with a pipeline spill on the environment. The effect of oil exposure on the visual learning of fish is important because vision is used for prey capture and predator evasion, which are both crucial factors in survival. In our study we examined the effect of exposure to these three types of oil on zebrafish (*Danio rerio*) visual learning behaviour. Zebrafish embryos were exposed to one of four treatments: mixed sweet blend, medium sour composite, dilbit, or embryo media (EM control). Mixed sweet blend, medium sour composite and dilbit treatments were made as a water-accommodated fraction (WAF) by mixing 1 part oil to 1000 parts water. Embryos were exposed from 0-7 days post-fertilization; at 7 days post-fertilization, larvae were shown a pre-recorded video containing 30 minutes acclimation, 5 minutes of visual stimulus followed by 5 minutes without, then another 5 minutes of stimulus. The larvae were recorded throughout and observed for behavioural change. By studying the effects of crude oils on visual learning behaviour we are better able to predict the more subtle consequences of a pipeline oil spill.

The effects of thyroid hormones, thyroid hormone disruptors, and treated wastewater on chemosensation in North American bullfrog tadpoles (PL)

Jody Heerema[1], Caren Helbing[2] and Gregory Pyle[1]

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Conventional wastewater treatment technologies were not designed to remove the multitude of everyday products, such as pharmaceuticals and personal care products, prevalent in wastewater today. Consequently, low concentrations of these products can persist in wastewater effluent after treatment. Some products can disrupt the normal functioning of the endocrine system in vertebrates even at low concentrations, and therefore are considered endocrine-disrupting compounds (EDCs). More specifically, some EDCs are thyroid active and therefore can interfere with the thyroid system by blocking or mimicking thyroid hormones (THs) thyroxine (T4) or tri-iodothyronine (T3). Tadpoles have been identified as excellent sentinels to study the effects of TH disruption because their development into adult frogs through metamorphosis is highly dependent upon the normal signalling of THs. With metamorphosis comes the major remodelling of the entire body plan, including the olfactory system. The tadpole olfactory system undergoes both structural and molecular changes as it transitions from functioning in a fully aquatic environment to functioning in both aquatic and terrestrial environments. As a result, the disruption of normal TH signalling has the potential to affect chemosensation during the sensitive developmental process of metamorphosis. This study aimed to measure the effects of TH disruption on chemosensation in North American bullfrog (*Lithobates catesbeianus*) tadpoles. Premetamorphic tadpoles were exposed to environmentally relevant concentrations of one of T3, T4, a cocktail of known EDCs, treated wastewater effluent or 17β-estradiol (E2) as a negative control. After exposures, avoidance responses to a chemosensory cue (amino acids) were measured using an I-maze behavioural assay. Our results show that T3 impairs chemosensory mediated avoidance responses to the cue, but T4 and E2 have no effect. Additionally the cocktail of known EDCs did not have an effect on the chemosensory behavioural responses, but treated wastewater effluent impaired the avoidance

responses. These results suggest that constituents in the wastewater effluent were able to mimic T3, as similar chemosensory mediated behavioural results were measured after both exposures. Healthy chemosensory acuity in tadpoles is important for them to locate food and avoid predators in order to reach the adult frog life-stage and reproduce. Therefore, chemosensory mediated behaviour can be a sensitive and ecologically relevant endpoint for studying the effects of TH disruption in tadpoles.

Ducks on drug soup (PL)

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Some mallard ducks may elect to overwinter on wastewater treatment ponds. These birds have been observed drinking from secondary clarified effluent (SCE) ponds. This water contains a diverse cocktail of pharmaceuticals and personal care products (PPCPs), pesticides and other contaminants. We first compared duck behaviour on these ponds to that on more typical overwintering sites and found no evidence of adverse effects. To determine directly if SCE ingestion could pose a health concern, we gavaged ducklings with SCE over days 2-30 post-hatch. We found both developmental and behavioural effects, but they were very subtle, and hard to characterize as adverse. For example, beak length was increased and pecking behaviour was reduced. Liver size was unaffected, which suggested the absence of a major toxic challenge. Additionally, we characterized a biotransformation enzyme, but its expression was not statistically increased by SCE exposure. Overall, our study suggests that ingestion of clarified wastewater may not pose a risk to overwintering waterfowl.

Toxicity of metal-contaminated sediments from the Upper Columbia River, Washington, to early life-stage white sturgeon (*Acipenser transmontanus*) (PL)

Edward Little[1]

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The upper Columbia River (UCR) has been contaminated by metal-loaded effluents resulting from smelter and mining activities. Approximately 10 million metric tons of slag, in addition to liquid effluent, were released from a lead-zinc smelter in Trail, British Columbia, from 1947 until 1995, resulting in some UCR sediment metal loads being elevated above biological criteria concentrations. In the transboundary reach of the UCR, white sturgeon (*Acipenser transmontanus*) population declines have been associated with little to no natural recruitment since the late 1960s. Copper, which is present in significant concentrations in slag, can be mobilized from slag-containing sediments and is highly toxic to early life-stage white sturgeon. Our objective was to evaluate toxicity of slag-contaminated sediments collected at various sites from the UCR to early life-stage white sturgeon and to assess various behavioural endpoints of early life-stage sturgeon to slag-contaminated sediments during exposure using field-collected and reference sediments. We exposed early life-stage white sturgeon to sediments from sites from the UCR in Reach 1, which were targeted for testing based on slag characteristics, copper concentration, and the potential for very early life-stage sturgeon to inhabit. Sturgeon were exposed for 14 days starting with 3 days post-hatch fish. Mortality was recorded and swimming activity endpoints were measured at the end of the exposure. In the overlying water, cumulative hardness-based chronic toxic units for the metal mixture of copper, cadmium, lead, nickel, and zinc were >1.0 for three of the six sediment treatments tested. The biotic ligand model normalized copper-only chronic toxic units were also >1.0 in three of the

six sediment treatments, although one treatment was different between the two measures. Mortality and swimming activity endpoints were significantly different from the quartz sand control treatment, with higher mortality and reduced activity measured in one sediment treatment which was collected from the UCR near Northport, Washington. This study sheds light on the cause-effect relationships of slag on the white sturgeon population.

Zebrafish shoaling behaviour is modulated by exposure to oil sands process water (PO)

Trevor Hamilton[1], Nathan Nadolski[1], Courtney Bailey[1] and Matthew S. Ross[1]

[1]MacEwan University

Oil sands process-affected water (OSPW) is a by-product of the recovery and separation of oil from surface mined bitumen. After its use in this process, OSPW is sequestered in large, lake-like storage basins called tailing ponds, until such time that it can be remediated and returned to the environment. In this study, we explored the possible effect of acute OSPW exposure on zebrafish (*Danio rerio*) behaviour. Zebrafish are an excellent model organism for this study because they exhibit basic and quantifiable behaviour that has been well documented in a variety of behavioural tests. Using replicate tanks for both groups, we exposed half of our fish (n=50) to a 10% OSPW environment and the other half (n=50) to dechlorinated Edmonton tap water as a control group for a period of 10 days. The fish were tested at the end of the 10-day OSPW exposure. To quantify locomotion and anxiety in the fish, motion tracking software was used to observe and analyze shoals of five zebrafish in an open field testing arena. The tendency of zebrafish to shoal is an innate response to predators or stress and is commonly used as an index of anxiety. Subsequently, the activity of the shoal was recorded after a novel object was introduced to the centre of the arena. In the OSPW-exposed fish, we observed a significantly larger inter-individual distance (IID) compared to our control group. Additionally, the duration and frequency that the OSPW fish spent in the centre zone of the testing arena were significantly greater. Taken together, these data suggest that short-term exposure to 10% OSPW alters shoal cohesion, indicative of decreased anxiety.

The impact of carbon dioxide induced freshwater acidification on zebrafish (PO)

Trevor Hamilton[1] and Dustin Newton[1]

[1]MacEwan University

Human generated (anthropogenic) carbon dioxide (CO_2) levels are on the rise. Many studies have shown that elevated carbon dioxide concentrations predicted for the year 2100 can have detrimental effects on marine organisms (commonly termed “ocean acidification”). In Californian rockfish, elevated carbon dioxide levels increase anxiety, and this is likely due to altered function of the GABAA receptor. There are similar changes that may occur in freshwater ecosystems causing acidification, yet this has rarely been investigated to date; however, studies in pink salmon do demonstrate alterations in behaviour. In this study we exposed zebrafish (*Danio rerio*) to approximately 3500 µatm of CO_2 and measured their behaviour. The novel tank diving test, a common measure of anxiety in zebrafish, was used after 4 days of elevated carbon dioxide exposure. To quantify the locomotion of the fish we used a motion-tracking software system and measured time in the top, middle, and lower zones of the arena, and total distance travelled. Here we will show the effects of elevated carbon dioxide levels on anxiety-like behaviour in zebrafish.

Effect of silver nanoparticles on alarm response in fathead minnows (*Pimephales promelas*) (PO)

William Dew[1], Andrew Darcy[1], Parastoo Razmara[2], Dan Rearick[1], Greg Pyle[2] and Maggie Xenopoulos[1]

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Fathead minnows (*Pimephales promelas*) undertake a fright response when presented with the skin-damage released chemical cue known as Schreckstoff. Response to this cue is dependent on an intact olfactory system, which can be impaired by a wide variety of organic and inorganic contaminants. Silver nanoparticles (AgNPs) have a variety of toxic effects on fish; however, to date no studies have been performed to determine how AgNPs affect the alarm response of fish. To determine if exposure to AgNPs affects the response of fish to alarm cue, fathead minnows were exposed to one of three concentrations of silver nanoparticles (0.17, 8.82, or 90 $\mu g \cdot L^{-1}$) or to control water for 48 hours. All exposure waters were made using filtered and ozonated Otonabee River water. The concentrations of AgNPs used in this study mirrored environmentally relevant concentrations as well as the concentration used in a whole-lake experiment in the Experimental Lakes Area as part of the Lake Ecosystem Nanosilver (LENS) project. Fish exposed to control water, 0.17, and 8.82 $\mu g \cdot L^{-1}$ AgNPs had an intact avoidance response to Schreckstoff, while fish exposed to the highest concentration (90 $\mu g \cdot L^{-1}$) did not respond to the alarm cue. A loss of response to alarm cue would put fish at increased risk for predation. Remarkably, fish exposed to 0.17 $\mu g \cdot L^{-1}$ AgNPs had an increased response to alarm cue as compared to control, indicating that the addition of a very low concentration of AgNPs somehow improved the olfactory ability of fathead minnows. It is unclear if this increased response would be beneficial or detrimental in an ecological context. Neurophysiological experiments are ongoing to determine if the effects of AgNPs on alarm response is due to a difference in olfactory acuity. These results demonstrate that AgNPs have an effect on the response of fathead minnows to alarm cue, however, more work is needed to determine the ecological consequences of an increased behavioural response due to the presence of 0.17 $\mu g \cdot L^{-1}$ AgNPs.

Community-driven Research in the North: Opportunities and Challenges

Northwest Territories-wide Community-based Water Quality Monitoring Program (PL)

Erin Kelly[1]

[1]*Government of the Northwest Territories*

During the development of the Northwest Territories (NT) Water Stewardship Strategy, Aboriginal community water partners expressed the desire and need to be actively involved in research and monitoring designed to address community questions about aquatic ecosystem health. Equally important was fostering capacity for communities to implement water quality monitoring over the long term. Questions about local water quality were generally in response to concerns about upstream development (particularly oil and gas, hydroelectric development and mining), climate change and municipal issues. The NT-wide Community-based Water Quality Monitoring Program started in 2012 to support the dual goals of answering local questions about water quality and building local monitoring capacity. Currently, community monitors in 21 communities, in collaboration with government technicians, collect water quality information at over 40 sites across the NT. Recognizing that there are many approaches to community-based monitoring, this presentation will highlight the development of the NT-wide Community-based Water Quality Monitoring program and outline key successes and challenges. The presentation will touch on program methodology and approaches, guiding community questions, mechanisms for capacity building and tools for information dissemination.

Slave River and Delta Partnership: Experiences and insights from a community-based monitoring program in the Northwest Territories (PL)

Rosy Bjornson[1], Jennifer Fresque-Baxter[2] and Slave River and Delta Partnership[3]

[1]*Deninu Kue First Nation,* [2]*Department of Environment and Natural Resources,* [3]*Slave River and Delta Partnership*

Understanding aquatic ecosystem health is important to community members that live along the Slave River and Delta. The river and its delta are important ecologically, spiritually, culturally and economically. The Slave River and Delta Partnership (SRDP) was formed in 2010 in response to community concerns about observed changes in Slave River and Delta, with the goal of working together to undertake coordinated research and monitoring about the health of this important area. The SRDP is a collaborative group consisting of Aboriginal, municipal, territorial and federal governments, academic institutions and Slave River and Delta community members. At the outset of the SRDP's formation, community partners identified three guiding questions that have shaped the group's research and monitoring efforts: (1) can we drink the water? (2) can we eat the fish and wildlife? and (3) is the ecosystem healthy? Simultaneously, community partners identified the critical need to build capacity at the local level to undertake research and monitoring and the importance of traditional and local knowledge underpinning all SRDP initiatives. This presentation will outline the history and development of the SRDP, guiding community questions and approaches to answering those questions, highlights of key research and monitoring initiatives, and key outcomes from the group's work. The presentation will also offer insights from our experiences that to help inform ongoing discussions about the critical importance of community-driven research and monitoring.

Participatory action research as community-driven research (PL)

Allice Legat[1]

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Community-driven research relies on communities to collaborate in a research process. Participatory action research, as conceived by the Dene Cultural Institute, is based on the premise that the community suggests and oversees the research, and participates in leading the resulting actions. Also important to participatory action research is the training of community researchers by both elders and the academics. Dene community members usually consider knowledge to be an individual entity that allows them to learn and share while building relationships in their community and beyond. Community members usually focus on understanding and taking action that is flexible enough to change when conditions necessitate. This paper considers two examples: “The Impact of Forest Fıres on Tǫdzı (Boreal Caribou)” research taking place in Whatì' in conjunction with the Wek'èezhìı Renewable Resources Board, Northwest Territories (NT), and “Finding Solutions to Health Impacts of Climate Change” research overseen by K'atłodeeche First Nation, NT. I found that community members want opportunities to share their knowledge while building capacity and relationships, and while providing their youth with a deeper understanding of living with the environment.

“One voice” to monitor northern Canada's freshwater aquatic environment: Method development to use Inuit traditional knowledge and western science in conjunction (PL)

Richard Nesbitt[1]*, Neil Hutchinson*[1]*, Luis Manzo*[2] *and Brenda Parlee*[3]

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Methods to assess environmental changes in the Arctic through scientific analysis are well established. However, vast, inaccessible expanses with a harsh climate present logistic and financial challenges that few other places in Canada experience. Community-based monitoring represents a potential to improve our ability to track changes in Canada's north, yet there is no established methodology to reconcile observations made by Inuit while on the land with scientific indicators such as water chemistry. Proponents have historically collected traditional knowledge as part of the Nunavut's environmental assessment process and use it to further justify establishing the freshwater environment as a “valued ecosystem component”. To date, however, no way to quantitatively use Inuit observations of the aquatic environment to track changes has been established, resulting in a reliance on patchwork scientific monitoring limited in both scope and scale. Qualitative observations are occasionally incorporated into environmental assessment documents, but there is a lack of consistency in both how that information is collected and how it is applied. We are currently developing a methodology to monitor the northern aquatic environment using Inuit traditional knowledge in conjunction with scientific observations. Development of the methodology is focused on trying to answer two core questions of community concern: “Is the water safe to drink?” and “Are the fish good to eat?” We are working throughout 2015-2017 to develop this methodology to bridge the two knowledge systems by conducting a series of interviews with traditional knowledge holders led by social scientists and uniquely attended by “curious scientists”. This allows, for the first time, targeted follow-up to historically superficial questions that overlook key variables that could be used to link western science with traditional knowledge. The intended outcome is to develop a set of common indicators between the two knowledge systems to characterize the aquatic environment, focusing on water quality and fish health. We are working to correlate, for example, the taste of high-quality drinking water sources as reported by

Inuit while on the land with chemical analysis, and observations of high-quality harvest species of fish with tissue analysis. Once refined, the method will increase the capacity of the community to be active participants in monitoring the aquatic environment and tracking cumulative impacts in their land use areas. It will facilitate a more consistent qualitative assessment of the environment and improve the use of Inuit traditional knowledge in the environmental assessment and other regulatory processes, thereby improving monitoring-based decision making at multiple jurisdictional levels.

Collaborative stream health monitoring in the Sahtu region (PL)

Julian Kanigan[1] *and Krista Chin*[1]

[1]*Government of the Northwest Territories*

This project aims to understand the impact of various disturbances on aquatic systems in an area of oil and gas exploration in the Sahtu region, in particular, for watersheds draining the eastern foothills of the Mackenzie Mountains. This project was stimulated by community concerns regarding oil and gas development taking place near their communities without an understanding of baseline conditions or how development may affect the aquatic environment and, in turn, their lifestyles. Currently, very little is known about factors that influence the health of the watersheds in this area. The landscape is impacted by natural (i.e., landslides and slumps) and anthropogenic disturbances (i.e., winter roads and seismic lines). There is a long history of oil and gas exploration in the area, which culminated in the horizontal fracturing of two well sites in February 2014. However, because of declining oil prices and the lack of transportation infrastructure, industry has left the region, allowing more time for the establishment of baseline information. This multidisciplinary project involves communities, regulators, government and academic researchers. The main objectives that will be discussed in this presentation are to: (1) build a partnership between communities, regulators, government and researchers; (2) measure water quality conditions and assess the health of stream ecosystems; and (3) contribute to community capacity-building in the Sahtu. We collected surface water and invertebrate samples from 47 sites. Data were collected in undisturbed sites, sites downstream from industrial development, landslides and slumps and areas impacted by 2014 and 2015 wildfires. Some sample sites were chosen by land users and elders, who identified areas that were important to them and their respective communities. Preliminary findings from 2013 and 2014 macroinvertebrate data demonstrated that there were no obvious trends in invertebrate abundance or composition with respect to a rudimentary classification of disturbances within the watersheds. A more refined system of measuring disturbances on the landscape will be used to analyze invertebrate abundance and community structure using data from 2013 to 2015. Since 2013, the project has supported 42 community person-days in the field and laboratory. Community technicians from Norman Wells and Tulita learned to collect data using the Environment Canada CABIN protocol, as well as identify and sort benthic invertebrates to the Order level. The project also contributed support to the Sahtu Renewable Resources Board for two annual 8-day cross-cultural research camps. Participants included 10 community environmental monitors-in-training, as well as 18 elders, harvesters and community members.

Research priorities in the Gwich'in Settlement Area (PL)

Kenneth Hunt[1]

[1]*Gwich'in Renewable Resources Board*

The Gwich'in Renewable Resources Board (GRRB) has established a research priority-setting process in the Gwich'in Settlement Area (GSA). This process includes the ongoing collection of research interests from the communities as one of its key criteria. The GRRB uses the end product to help set its work plan and make decisions on project funding. Examples can be given of community-based monitoring programs that are closely linked to community interests with full involvement of the community. The research interests from the communities include research that is not related to the GRRB's mandate but could be of interest to other researchers. The GRRB is interested in finding other ways to advertise these interests to promote research that addresses them.

Understanding and predicting fish mercury levels in the Dehcho region (PL)

Heidi Swanson[1]*, George Low*[2]*, Brian Branfireun*[3] *and Norma Kassi*[4]

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Many northerners are concerned about levels of mercury in food fishes such as northern pike (*Esox lucius*), walleye (*Sander vitreus*), and lake trout (*Salvelinus namaycush*). In some traditional fishing lakes, mercury levels are high enough to have led to consumption advisories. Fishers, community members, regulators, monitors, and scientists want to understand why fish mercury levels are relatively low in some lakes but higher in others, and why fish mercury levels are increasing in some lakes but stable in others. By understanding the dominant drivers of fish mercury in the Dehcho, we can more accurately predict how climate change and resource development may affect fish mercury. In this presentation, we will discuss challenges, successes, and lessons learned (so far) during two community-driven research projects on understanding and predicting fish mercury levels in northern lakes. In one study, a joint university and community research team completed an intensive one-year study on Kluane Lake, Yukon. This project formally included traditional knowledge interviews with elders, collaboration with subsistence fishers, and an intensive youth training component. Results of the study indicated that mercury levels in food fishes were remarkably low, and thus communication and analysis of results was very straightforward. In the second much more complex study, eight lakes in Dehcho region of the Northwest Territories were sampled over three years. This project involved several different First Nations, a joint university-Indigenous environmental monitor team, and an informal youth component. Results of this study have led to very interesting community-driven management initiatives, such as fish-downs. Results indicate that monitoring certain water chemistry parameters in these Dehcho lakes will be useful in predicting the trajectory of mercury in some food fish species.

Linking Molecular, Physiological and Behavioural Responses Following Exposure to Endocrine-Disrupting Chemicals

THC exposure during gastrulation alters heart rate and neuronal morphology in zebrafish (PL)

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The psychoactive ingredient in marijuana is delta-9-tetrahydrocannabinol (Δ9-THC). Marijuana, derived from the plant *Cannabis sativa* L., is commonly used for medicinal purposes but is also used as an illicit recreational drug. In western societies, it is often taken recreationally along with alcohol and may pose a significant risk to embryonic development as it freely crosses the placenta. In fact, THC exposure during development has been shown to lead to deficits in fine and gross motor control. Here, we used zebrafish (*Danio rerio*) embryos to determine the effects of THC treatment during gastrulation. We treated embryos in the gastrulation stage (5.25 hours post-fertilization (hpf) to 10.75 hpf) with 2 $mg{\cdot}L^{-1}$, 4 $mg{\cdot}L^{-1}$ or 6 $mg{\cdot}L^{-1}$ THC and examined the effects on animal morphology, heart rate, Mauthner cell (M-cell) morphology and the c-start escape response. THC (6 $mg{\cdot}L^{-1}$) treated fish were smaller than controls with a body length of 3.140.02 mm (n=30) compared with untreated animals 3.23±0.01 mm (n=25) ($p<0.05$). THC treatment also induced a reduction in heart rate. Exposure to 6 $mg{\cdot}L^{-1}$ of THC resulted in embryos with a heart rate of 85±3 (n=42) beats per minute, while untreated animals had a heart rate of 124±2 (n=43) beats per minute ($p<0.01$). Mauthner neurons are large reticulospinal neurons associated with the c-start escape response in teleosts. These cells are born at 8 hpf, during gastrulation and during the THC exposure period. Therefore, we compared the M-cells of treated and untreated fish to ascertain if there were any morphological differences between the groups. We found that THC treated animals had significantly smaller M-cell axon diameters (1.25±0.05 µm; n=10) compared with untreated fish (1.94±0.14 µm; n=8) ($p<0.001$). Because the M-cells are involved in the c-start escape response, we examined locomotor characteristics of the escape response. We found that THC treatment induced an increase in the initial angle of tail bend (323±13 degrees; n=12) compared with controls (216±15 degrees; n=15) ($p<0.05$), but no significant change in the peak acceleration or peak speed of the tail movement. Together, these findings indicate that THC exposure during gastrulation can lead to alterations in heart rate, neuronal morphology and some aspects of locomotion in embryos.

A combined transcriptomic and proteomic approach to identify toxicity pathways in early-life-stages of Japanese medaka (*Oryzias latipes*) exposed to 1,2,5,6-tetrabromocyclooctane (TBCO) (PL)

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1,2,5,6-tetrabromocyclooctane (TBCO) is a novel brominated flame retardant considered as a potential replacement for the major use flame retardant hexabromocyclododecane (HBCD). Recent studies in our lab have demonstrated that TBCO is an endocrine-disrupting chemical. TBCO alters

estrogen (ER) and androgen (AR) receptor signalling, and synthesis of estradiol (E2) and testosterone (T), *in vitro*. Also, exposure to TBCO impairs reproductive performance of Japanese medaka (*Oryzias latipes*), and this effect appears to be multi-generational. To gain a greater understanding of effects of this novel chemical, RNA sequencing and proteomics were used to characterize responses of transcriptome and proteome in embryos of embryos of medaka exposed to waterborne TBCO. Several putative toxicity pathways that likely are independent of the endocrine-disrupting effects of TBCO were identified and were confirmed by use of bioassays. However, results of the omics analyses did provide information about potential mechanisms of multi-generational effects of TBCO on reproduction. This work not only increased understanding of toxicity of TBCO, including its endocrine-disrupting effects, it also demonstrated that simultaneous use of transcriptomics and proteomics provides a comprehensive understanding of potential mechanisms of toxicity of chemicals, and can be used in concert to explain and predict apical effects on attributes that contribute to fitness.

Development of an adverse outcome pathway for neurobehaviour in several different species of larval fish to predict ecological impacts from molecular responses (PL)

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The adverse outcome pathway framework (AOP) is an effective collaborative platform to unite multidisciplinary scientists to focus on challenging ecological problems, to form hypotheses and direct research. In our work, we focus on behaviour, which is a challenging endpoint to incorporate into ecological risk assessment because it is difficult to infer how subtle changes in behaviour translate to ecological metrics (such as population abundance trajectories), and it is often difficult to setup appropriate behavioural assays in the laboratory (especially for species not typically maintained in the lab). But behaviour of organisms, if measured appropriately in laboratory assays, can provide much integrated insight into the physiological status of the individual, with implications for key demographic parameters such as individual survival and growth rates. We use the AOP framework to unite several different technologies and approaches: behavioural assays, gene expression, metabolomics, individual based models, and risk analyses to predict the effect of contaminants across levels of biological organization and infer population responses to quantify risk of exposure to certain chemicals. By using the AOP framework in a systematic way, we collect data on multiple species and facilitate cross-species comparisons. We also collaborate with other agencies and institutions that do similar work on different species, which assists our cross-species comparisons. Our aim is to determine what information can be collected on model species that will be informative for behavioural impairments and population responses on other species. We also wish to determine if molecular information can substitute for behaviour assays related to foraging success and learning, as such assays are labor intensive and challenging to do on non-model species and molecular-level information is relatively easier to collect. The AOP framework has been immensely helpful for organizing, focusing and propelling this research forward.

The pharmaceutical fluoxetine as endocrine disruptor in teleost fish (PL)

Jan Mennigen[1]

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Fluoxetine is an antidepressant pharmaceutical which is routinely detected in effluent-receiving watersheds in the low ppm range ($\mu g \cdot L^{-1}$) as a consequence of incomplete retention in urban wastewater treatment plants. Fluoxetine has been shown to bio-concentrate in wild fish, resulting in measurable quantities of fluoxetine and its active metabolite, nor-fluoxetine, in several tissues. This has led to concerns of organismal-level physiological consequences in response to sublethal exposure to fluoxetine, especially since additional (antidepressant) pharmaceuticals with similar modes of action are also routinely detected in effluent-receiving water systems, raising concerns of potentially synergistic effects. In this platform session, I will present evidence from integrative studies using the goldfish *Carassius auratus*, a model organism in comparative endocrinology, which identify fluoxetine as an endocrine-disrupting chemical. Specifically, the disruptive effects of fluoxetine, including environmentally relevant concentrations, on (neuro)-endocrine systems regulating reproduction and energy metabolism in goldfish will be discussed. Additional relevant evidence from studies in other fish species and aquatic invertebrates will be presented to highlight how molecular, physiological and behavioural endpoints have been used to identify endocrine-disrupting properties of fluoxetine and other antidepressant pharmaceuticals detected in the aquatic environment.

Development and function of the cortisol stress axis: A marker of performance disruption in fathead minnows (PL)

Bastien Sadoul[1]*, William Thompson*[1]*, Patrick Gauthier*[1] *and Mathilakath Vijayan*[1]

[1]*University of Calgary*

Municipal wastewater effluents (MWWEs) are carrying a wide range of human-made molecules with individually well-established adverse effects in wildlife. Describing the biological effects of MWWEs on native species may provide novel biomarkers of health and performance disruptions for environmental effects monitoring. The stress response is playing a central role in allowing the animal to cope with a new environment, including predator avoidance and ultimately survival in stressful situations. However, there is a paucity of information regarding the ontogeny of the stress axis in native fish species, including the fathead minnow (*Pimephales promelas*). We hypothesize that early life exposure to MWWEs disrupts the proper development of the cortisol stress axis, leading to reduced fitness in fathead minnows. The development and function of the cortisol stress axis was characterized by temporal measures of whole-body cortisol content, as well as cortisol response to a stressor at critical stages during early development. Also, the behavioural response to a stressor, a sudden light pulse, was monitored during embryogenesis in this species. The results suggest that key stages of the stress axis ontogeny may be targets to assess the effects of contaminants, either singly or as mixtures. Overall, the results provide a general framework to study the effect of emerging contaminants in MWWE on the functioning of the endocrine stress axis in fathead minnows. The study was funded by the Natural Sciences and Engineering Research Council of Canada Strategic Grant to MMV.

The effects of endocrine-disrupting chemicals singly and in mixture on developing zebrafish (PL)

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Exposure to environmental contaminants has been linked to developmental and reproductive abnormalities leading to infertility, spontaneous abortion, reduced number of offspring, and metabolic disorders. In addition, there is evidence linking environmental contaminants and endocrine disruption to abnormal developmental rate, defects in heart and eye morphology, and alterations in behaviour. Notably, these effects could not be explained by interaction with a single hormone receptor. We used a whole-organism approach to investigate morphological changes to developing zebrafish (*Danio rerio*) caused by exposure to a number of environmental contaminants, including bisphenol A (BPA), di(2-ethylhexyl)phthalate (DEHP), nonylphenol, and fucosterol at concentrations measured in a local water body (Oldman River, Alberta), individually and in mixture. In this presentation, I will demonstrate that exposure to nanomolar contaminant concentrations resulted in global developmental changes, as well as abnormal pericardial (heart) development and abnormal head development. In addition, we examined the effects of contaminants singly and in mixture and show that experimental mixtures caused different phenotypes than theoretical mixtures. Combined, our results support the hypothesis that adverse effects of contaminants are not mediated by single hormone receptor signalling. Furthermore, our results support the view that adversity of contaminants in mixture could not be predicted by simple additive effect of contaminants. The findings provide a framework for better understanding of developmental toxicity of environmental contaminants in zebrafish and other vertebrate species.

Maternal transfer of bisphenol A causes generational toxicities in zebrafish (PL)

Jith Kallarakavumkal Thomas[1] and Mathilakath Vijayan[1]

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Bisphenol A (BPA) is widely prevalent in the environment and there is mounting evidence of its impact on the endocrine systems of animals. Although an environmentally realistic concentration of BPA is shown to cause endocrine dysfunction, less is known regrading generational toxicities of this chemical in fish. Recent studies suggest that BPA exposure can lead to developmental effects in fish, but the mechanisms are far from clear. The objective of this study was to investigate persistent and generational toxicities of maternally deposited BPA in zebrafish (*Danio rerio*). Zebrafish embryos were microinjected with 0, 1, 10 or 100 pg BPA per embryo to mimic a maternal transfer scenario of this chemical to the embryo. The impacts of BPA on development, behaviour and acute stress-response in the early life-stages as well as adults in the F1, F2 and F3 generations were determined to investigate the long-term adverse effects of maternally deposited BPA in fish. Embryo accumulation of BPA modified the development and locomotor behaviour in early life-stages of F1 and F2 generation fish. There were no differences in cortisol production in fish from control and BPA-exposed treatment groups. Reproductive capacities (egg production, hatchability and/or embryo survivability) were altered in F1 generation adult fish from 1 and 10 pg BPA-exposed groups but not in 100 pg BPA group, suggesting a monotonic dose-response for BPA-induced reproductive toxicities in fish. There were no differences in swimming performance and avoidance temperature in adult fish from the control and BPA-exposed treatment groups. Overall, our results suggest that BPA affects multiple aspects of fish performance and some of

these changes are evident even in multiple generations in zebrafish. The study was funded by the Natural Sciences and Engineering Research Council of Canada Strategic Grant (to Vijayan).

Molecular and behavioural responses of caged goldfish to pharmaceuticals and personal care products exposure in Cootes Paradise (PL)

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[1]University of Waterloo, [2]Environment and Climate Change Canada, [3]McMaster University, [4]AXYS Analytical Services Ltd.

Cootes Paradise is a wetland on the western tip of Lake Ontario, and is part of the Hamilton Harbour Area of Concern. Cootes Paradise is also an important site of biological diversity and a fish nursery. The primary source of contaminants in Cootes Paradise is the Dundas, Ontario, municipal sewage treatment plant (STP), which discharges into the marsh. Pharmaceuticals and personal care products (PPCPs) have been associated with STPs and their recipient waters. Some PPCPs are known to cause endocrine disruption. To assess the endocrine-disrupting potential of Dundas STP effluents to wild fish in Cootes Paradise, male goldfish were caged for 21-days (5 individuals per cage, 10 cages per site) at three sites along a contamination gradient within Cootes Paradise. We also caged fish at a reference site in Lake Ontario: Jordan Harbour. Twenty male wild goldfish were captured from Cootes Paradise in the summer of 2012. Concentrations of PPCPs and profiles of endogenous proteins and metabolites were determined in plasma from both the caged and wild goldfish. Half of the caged fish were assessed in behavioural assays. This talk will (1) describe the observed behavioural changes and the protein and metabolite expression patterns of the caged fish, (2) relate those changes to the burden of PPCPs in the fish plasma, and (3) assess the ability to predict from caged fish whether similar effects are likely in wild fish populations.

Mining and the Environment

Challenges with toxicity testing at an Arctic diamond mine (PL)

Alexandra Hood[1]

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De Beers Canada Inc. owns and operates the Snap Lake Diamond Mine, which is located 220 km northeast of Yellowknife, Northwest Territories. Regulatory approvals were granted in 2004 and the mine commenced operations in 2007. In 2012, the Mine's water licence was renewed for a period of eight years. In 2013, an application was submitted to the Mackenzie Valley Land and Water Board to amend this licence to include changes to water quality parameters. Under the Mine's water licence, there is a requirement to complete quarterly acute toxicity tests on mine effluent for rainbow trout (*Oncorhynchus mykiss*) and *Daphnia magna*, and chronic toxicity tests for *Ceriodaphnia dubia* and *Pseudokirchneriella subcapitata*. The Mine also completes two annual toxicity tests on water collected from within the in-lake water discharge mixing zone, including an early life-stage (ELS) test with rainbow trout, and a 7-day test of larval growth with fathead minnow (*Pimephales promelas*). The chronic toxicity test for *C. dubia* and ELS test for rainbow trout have proved challenging for the Mine due to a number of factors. In 2014, the water quality Low Action Level for toxicological impairment was triggered following observed effects on *C. dubia* reproduction in two concurrent samples. Periodic recorded impairment to the waterflea, *C. dubia*, during the test has occurred inconsistently since 2005; there is no correlation to water chemistry or other toxicity tests, and resident waterflea populations are healthy. The long-term monitoring program in the lake indicates that there have been changes in resident aquatic communities in Snap Lake; however, these changes do not reflect the results from laboratory toxicity testing with *C. dubia*. An inter-laboratory study resulted in surprising variability between three accredited toxicity testing laboratories, which may at least partly explain some of the previous variability in toxicity test results. Regulatory scrutiny and pressure for action and follow-up investigations have increased although the evidence suggests false positives rather than early warning for Mine effects. For the rainbow trout ELS test, lack of availability of gametes and failures within the control groups has occurred repeatedly. As well, there is only one accredited laboratory in Canada equipped to conduct this test. Logistically, collecting water for the test is challenging as gametes are often only available when it is unsafe to traverse the lake, and collection and shipment of test samples is often impacted by weather on site. Issues with hold times associated with shipment from a remote location by plane have further confounded testing. This presentation will present and consider the challenges experienced at the Snap Lake Diamond Mine related to these toxicity tests, and will consider potential implications and paths forward for these two toxicity tests at the Mine and other remote sites.

The benefit of a strong study design in metal mining environmental effects monitoring: Case study from AREVA's McClean Lake operation (PL)

Sarah Benson[1]*, Arden Rosaasen*[1] *and Bruce Kilgour*[2]

[1]*AREVA Resources Canada Inc.,* [2]*Kilgour & Associates Ltd.*

AREVA Resources Canada Inc. operates a uranium mine and mill at its McClean Lake operation in northern Saskatchewan. The mine/mill operation is subject to the federal Environmental Effects Monitoring (EEM) program, in addition to provincial and Canadian Nuclear Safety Commission (CNSC)

monitoring requirements. To address the provincial and CNSC requirements, the mine/mill developed a robust monitoring study design for benthic invertebrate communities in a downstream lake environment, based on an “optimal” before-after-control-impact (BACI) study design. Control-impact study designs are the “default” design option in the federal EEM program. Further, statistically significant differences in indices of composition between reference and exposure areas are what are used in the EEM program to indicate effluent-related “effects”. Control-impact designs are highly vulnerable to demonstrating natural differences. The data from AREVA's robust BACI demonstrate that simple control-impact designs can lead to erroneous conclusions about effects. Further, this data set illustrates the challenge of focusing on statistical differences that are within background ranges of normal variability.

Improving mining assessment and monitoring (PL)

Peter Chapman[1]

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Mining activities are no different from any other human activities—they cause physical changes to the environment and chemical changes to the waters into which effluent is discharged. The purpose of environmental assessments (EAs) is to determine the extent of those changes such that regulators and communities of concern can determine whether mining should be allowed to proceed. However, EAs are not certain; unexpected changes can and will occur, not always related to mining. Further, assessing the effects of mining can be complex given natural variability, climate change, and invasive species. In addition, changes are not always negative; they may be neutral or even positive. Key improvements for assessing and monitoring mining activities include: a minimum of three years of comprehensive baseline studies to attempt to bound natural variability; explicit recognition that meeting EA predictions is not always possible or necessary; regulatory control that is flexible rather than overly prescriptive; a primary focus on resident biota such that those data have primacy over laboratory analyses or tests; a clear focus on obtaining essential information, not “nice to have” information; revisions to monitoring programs over time that allow for deletions not just additions; and, allowance for common sense. Real-life examples will be provided to illustrate these recommendations.

Development and standardization of new saline effluent toxicity test methods using marine fish and invertebrate species (PL)

Rick Scroggins[1], *Lisa Taylor*[1], *Leana Van der Vliet*[1] *and Ryan Hennessy*[1]

[1]*Environment and Climate Change Canada*

The *Metal Mining Effluent Regulations* (MMER) under the *Fisheries Act* requires that the final effluent from all metal mines be non-acutely lethal to fish. Currently, the acute lethality Reference Method prescribed in the MMER, as a compliance requirement, requires the use of juvenile rainbow trout (*Oncorhynchus mykiss*) as the test species (EPS 1/RM/13); and the invertebrate method, prescribed for acute toxicity monitoring, requires the use of *Daphnia magna* (EPS 1/RM/14). In an upcoming amendment of the MMER, Environment and Climate Change Canada (ECCC) will be changing the purpose of the *D. magna* test from monitoring-only to a separate compliance parameter for measuring acute lethality. Until recently, there was no need to consider how to test the acute lethality of saline metal mining effluents in Canada, as only freshwater mining effluents existed. The mining of northern ore deposits within saline groundwater zones is now a reality and has created the need for ECCC to

develop new marine toxicity tests for measuring the acute lethality of saline effluents that will be discharged to estuarine or marine receiving environments. In time for the upcoming amendments of the MMER, ECCC must: identify an appropriate marine fish and invertebrate test species; standardize the holding or culturing conditions plus test design; validate the new methods through an inter-laboratory validation study; and write the formal Reference Methods. ECCC will standardize and publish the new test methods while working with Canadian toxicology laboratories so they are prepared to offer testing services to mining companies and other industries with saline effluent discharges (e.g., fish and food processing plants). This presentation will cover our progress to date and the detailed plan to completion of the new Reference Methods for measuring acute lethality of saline effluents.

Natural concentrations of manganese appear to cause mortality in two standard freshwater sediment tests: A case study of four pristine lakes in northwestern British Columbia (PL)

Ryan Hill[1], Brett Lucas[2], James Elphick[2], Eric Franz[1] and Gary Mann[1]

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Sediments from four pristine lakes situated in a mineralized region of northwestern British Columbia were tested in 2014 using a 28-day *Hyalella azteca* survival and growth test, and a 10-day *Chironomus dilutus* survival and growth test. The lakes were sampled as part of baseline studies for a proposed mine development. Previous bulk sediment sampling conducted in 2013 had identified a number of metals naturally exceeding provincial sediment quality guidelines in each lake. The 2014 program included synoptic sampling for bulk sediment chemistry (metals, pH, total organic carbon, particle size, acid volatile sulfide (AVS), simultaneously extracted metals (SEM)) and was conducted to establish baseline toxicological conditions as a point of comparison for any future toxicity testing. Three sediment samples, each a composite of approximately three Ekman grabs, were collected from each of the four lakes. In the lab, five replicates were tested for each of the 12 samples, and each replicate was initiated with 10 organisms. Peepers were inserted into a sacrificial replicate of the *Chironomus dilutus* test for passive subsampling of metals in porewater. Survival for both tests was impacted in four of the 12 samples, in most cases severely. Among metals measured in bulk sediments, three (iron, manganese, nickel) were above sediment quality guidelines and were generally correlated with the survival endpoints in both tests. Subsequent comparison of the survival endpoints with porewater chemistry from the peepers showed strong correlations for manganese and nickel only. Nickel concentrations were orders of magnitude lower than concentrations associated with acute effects on these two taxa reported in the literature. Manganese concentrations in porewater in the four highly impacted samples were higher than reported LC_{50} values in the literature for waters of low hardness. Thus, it seems likely that mortality was caused by dissolved manganese, mobilized under reducing conditions associated with decomposition of organic matter. Further laboratory work to confirm the cause of toxicity was not pursued, because the effects were associated with naturally occurring conditions. All of the impacted samples were from the two deepest lakes, where manganese concentrations in sediment and porewater were highest. We conclude that these tests, at least in the deeper lakes, would not be useful as a future monitoring tool if mine development proceeds.

Sulfate effects on selenium accumulation by algae: Extrapolating from lab to field (PL)

Adrian deBruyn[1], Jordana Van Geest[1], James Elphick[2] and Bonnie Lo[2]

[1]Golder Associates Ltd., [2]Nautilus Environmental

Selenium release from waste rock is an environmental concern at many coal, base metal, and precious metal mines across Canada that can persist well beyond closure. Effective assessment and management of this concern requires a site-specific understanding of selenium fate and bioaccumulation. A key element of this understanding relates to sulfate, which reduces selenate uptake by algae and can thereby decrease the exposure of all trophic levels. We combined laboratory and field studies to characterize the sulfate dependence of selenium bioaccumulation at the base of aquatic food webs in surface waters where sulfate and selenium concentrations are correlated. A laboratory study was conducted to model the effect of sulfate on selenium uptake kinetics and steady-state selenium bioaccumulation in algae under controlled conditions. Analysis of uptake kinetics confirmed that tests had achieved steady-state, allowed evaluation of whether effect of sulfate occurs via changes to selenium uptake, elimination, or both, and allowed growth dilution to be quantified so that test results could be more directly related to field data. These results were then combined with field data from coal-mining regions in British Columbia, Canada. This lab-to-field integration involved characterizing the combined effect of covarying aqueous selenium and sulfate concentrations, and accounting for differences in algal growth between lab cultures and natural streams. The resulting "hybrid" model integrated laboratory and field data to show how sulfate affects selenium bioaccumulation at the base of natural aquatic food webs.

Effects of elevated salinity and dissolved organic matter in surface water from an oil sands mine end-pit lake on the toxicity of metals to zooplankton (*Ceriodaphnia dubia*) (PL)

Kevin White[1] and Karsten Liber[1]

[1]University of Saskatchewan

In order to manage growing inventories of fluid fine tailings (FFT) and oil sands process-affected water (OSPW), Alberta oil sands mine operators have proposed end-pit lakes (EPLs) as a method for the long-term storage and reclamation of tailings. In December 2012, Syncrude began the industry's first large-scale demonstration of EPLs with the creation of Base Mine Lake (BML), an artificial lake containing FFT capped with OSPW and fresh water. However, tailings contain a complex mixture of dissolved organics, salts and metals which have adverse effects on zooplankton, aquatic organisms essential for early ecosystem development. The initial elevated salinity of BML decreased rapidly during 2013 and 2014, but has since slowed; chloride (410 $mg \cdot L^{-1}$) is now within a tolerable range for most freshwater organisms, but will likely still cause stress. From October 2014 to August 2015, most dissolved metals concentrations decreased an average of 60%. Copper (Cu) and zinc (Zn) were the only metals which increased during this time (>300% and >200%, respectively). As of August 2015, Cu is the only metal of concern which still exceeds Canadian Water Quality Guidelines for the Protection of Aquatic Life (arsenic (As), cadmium (Cd) and chromium (Cr) exceeded in 2014), although some metals remain highly elevated above background levels (molybdenum (Mo) is 45x higher in BML than in the Athabasca River). Based on an initial risk assessment and chronic toxicity hazard quotients, the concentrations of As, Cd, Cr, cobalt (Co), Cu and nickel (Ni) in BML were all identified as being of potential concern to initial aquatic ecosystem colonizers such as *Ceriodaphnia dubia*. Current testing is focused on characterizing the

toxicity of these metal mixtures to *C. dubia* and the potentially attenuating effects of the elevated salinity and dissolved organics present in BML surface water.

Consideration of environmental effects in setting acceptable release limits (PL)

Stacey Fernandes[1], Katherine Woolhouse[1] and Caroline Lucas[1]

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The potential effects on ecological receptors can be used as the basis for defining the maximum acceptable release of effluent from industrial facilities such as a mine, manufacturing plant, or waste management facility. Exposure-based Release Limits (EBRLs) are effluent release loads that have been set based on selected levels of protection to the receiving environment. For releases to water, consideration can be given to effects on water and sediment quality, as well as the protection of aquatic biota (e.g., fish, invertebrates), semi-aquatic wildlife (e.g., ducks, muskrat), or human health. Predictive models that consider contaminant movement through watershed systems and the food chain can be useful tools for setting EBRLs by allowing the back-calculation of the effluent release load based on meeting selected concentrations in various environmental media. One of the key elements for the derivation of EBRLs is the selection of pertinent ecological receptors and the appropriate ecological toxicity values. Other factors that need to be considered in the setting of EBRLs include the location at which the protection levels apply (i.e., point of compliance), the considered timeframe (i.e., current or future conditions), and whether the EBRL should be based on expected or upper bound conditions. Stakeholder consultation, including input from Indigenous communities, can be used to guide these decisions. Examples of EBRLs derived for a hypothetical mining operation will be provided. This will include discussion of the dispersion within the aquatic environment (rivers, lakes and wetlands), fate and transport through the aquatic food chain, and intake by wildlife and human receptors. The sensitivity of EBRLs to site-specific environmental factors will be discussed as well as the differences in constraining factors for derivation of EBRLs for contaminants. Once derived, the EBRLs can be used by the facility operator for planning purposes in the determination of effluent quality criteria, which would consider other factors such as regulatory limits, technology-based release limits, pollution prevention, and cost-benefit analysis.

Environmental study of a mine 25 years after closure (PL)

Carolyn Brown[1], Karen Petersen[1] and Brian Fraser[1]

[1]EcoMetrix Incorporated

An extensive environmental study was recently completed at a closed mine in central Ontario. The study included the assessment of site aspects (waste rock stockpiles, tailings, water-filled pits) and an evaluation of water quality, sediment quality, and biological communities (benthic invertebrates) on-site and downstream. Models were developed to describe ongoing recovery of sediments in the downstream receiving environment, and risks to downstream aquatic receivers were evaluated to guide ongoing site management and potential rehabilitation strategies. On-site sources of acid rock drainage / metal leaching were largely mitigated by the filling of the open pits with water. Four of the five water-filled open pits are deep and are permanently layered as a result of higher density waters below 20 metres. There are elevated concentrations of iron and manganese in the deeper layers, but those layers are isolated from and do not mix with the upper layers, so they are not expected to affect downstream

water quality. The lower concentrations and neutral pH values in the upper layers of pit waters, which represent the outflows from the pits, approach or are better than provincial water quality criteria. The quality of water leaving the site meets provincial water quality criteria. The concentrations of constituents of potential concern in lake sediments were found to be elevated with respect to background levels. Sediment core samples provide a history of changes of concentrations over time. The results clearly showed that metal concentrations in all on-site lake sediments increased from background levels during the mining operation as expected, and then stabilized before declining after mine closure in response to declining concentrations in water at the site. Sediment quality modelling suggests sediment recovery will be ongoing for some time. Despite relatively high constituent levels in downstream sediments, no differences were seen between the benthic communities downstream of the site and at reference areas, indicating that mine-related effects do not extend off-site.

Multiple years of non-lethal mercury monitoring in lake trout at the Diavik Diamond Mine (PL)

Tamara Darwish[1], Clayton James[1], Hilary Machtans[1] and David Wells[2]

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In 2007, a slimy sculpin (*Cottus cognatus*) survey in Lac de Gras, Northwest Territories, revealed that body burdens of mercury were significantly greater in a population exposed to treated diamond mining effluent, compared to those of two reference populations within the same lake. To determine whether mercury increases were occurring in the larger edible fish of Lac de Gras, a lake trout (*Salvelinus namaycush*) mercury monitoring program was implemented in 2008 and repeated in 2011 and 2014. Mercury concentrations were measured in lake trout captured in two connected lakes, Lac de Gras and Lac du Sauvage, using non-lethal sampling methods. Non-lethal sampling methods provide an accurate alternative to the traditional destructive fillet sampling technique, which can impact the older, slower-growing lake trout populations found in the North. Mercury concentrations were found to be increasing from 2008 to 2011 in Lac du Sauvage, but not in Lac de Gras, where the Diavik Diamond Mine is located. A movement study in 2014 confirmed that the lake trout move between the two lakes, indicating that captured lake trout used for the study could have originated from either of the two lakes. In 2014, mercury concentrations decreased in both lakes and were found to be at concentrations similar to those observed prior to treated effluent release in Lac de Gras. The cause of the decrease in mercury is unknown.

Surface water quality monitoring under the Joint Oil Sands Monitoring Program in the lower Athabasca River and downstream receiving environments (PL)

Kerry Pippy[1] and Nancy Glozier[1]

[1]Environment and Climate Change Canada

The water quality monitoring program outlined by the Joint Canada-Alberta Implementation Plan (JOSMP) was implemented in 2012. The Plan was designed to address recommendations for the development of an integrated “state-of-the-art” monitoring program which would be able to identify changes in environmental condition over time in Canada's oil sands (OS) region. Of the 21 current monitoring sites reported through JOSMP, only five had pre-existing water quality monitoring programs in operation. Routine water quality sampling approaches were reviewed, streamlined, and standardized,

resulting in data from over 1300 samples for more than 270 parameters (nutrients, major ions, total and dissolved metals, total and methylmercury and organics) representing over 240,000 measurements—a nearly five-fold increase in sampling effort. Several non-standard monitoring approaches were evaluated including detailed cross-sectional sampling, auto-monitoring deployments, and semipermeable membrane devices (SPMDs) for the collection of time-integrated low level organics. Status and trend results are reported for Athabasca, Peace and Slave rivers as well as multiple tributaries and interconnection channels of the Peace Athabasca Delta. Concentration in the larger rivers showed more similarity when compared to the smaller tributaries. Evaluation of the cross-sectional sampling found high cross sectional variability (more than 50%); however, no significant difference in loading estimates was determined from the range of sampling approaches tested. Auto-monitoring approaches look promising but design issues in remote locations were a challenge. Evaluation of the data against available water quality guidelines revealed that nineteen of the parameters (alkalinity, pH, 2 nitrogen nutrients, 5 total metals, methylmercury and 9 organics) showed no exceedance. Total metals commonly (50-90%) showed values higher than the guideline, particularly during periods of high suspended sediment concentrations. Total mercury showed occasional (less than 6%) excursions and, similar to total metals, these values were associated with high suspended sediment values. All other parameters had excursion rates less than 20%. Long-term trends were updated for the monitoring site on the Athabasca River near Wood Buffalo National Park. Most trend results were similar to those previously reported, but an interesting exception was those for total and dissolved phosphorus. At this site, the increasing trend in total phosphorus appears to have been curtailed, and dissolved phosphorus concentration has been declining over the last 15 years.

Data methods and quality control measures for a passive water quality monitoring program in the oil sands region of Alberta, Canada (PL)

Cari-Lyn Epp[1], Lucie Levesque[1] and Nancy Glozier[1]

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Environment and Climate Change Canada (ECCC), in partnership with the Province of Alberta, implemented a passive water quality monitoring program in support of long-term water quality monitoring in the oil sands region of Canada. The monitoring program employs semi-permeable membrane devices (SPMDs), which passively accumulate dissolved organic compounds for the estimation of time-weighted concentrations of potentially toxic, bioaccumulative polycyclic aromatic compounds (PACs). Semi-permeable membrane devices were deployed monthly from 2013 to 2015 at up to seven sites along the Athabasca River. Concentrations of PACs were measured from membranes that were deployed in the water, as well as from an extensive suite of quality control membranes (field blanks, dialysis (manufacture) blanks etc.). These concentrations were used to examine the relative frequency, magnitude and variation in PAC concentrations in the environment relative to those present as background (i.e., within blanks). Field blanks accounted for approximately 40% of the 112 samples collected. Target analytes in field blanks were less frequently detected, were lower in magnitude, and less variable than those in water samples. Concentrations were in many instances indistinguishable between field and dialysis blanks, and were determined to be affected by systematic drivers (e.g., manufacture lot and storage time). In order to examine these further, more than 50 dialysis blanks were analyzed to quantify variation in background concentrations in membranes (replicate n=6-16) upon manufacture, between analytical batches, and subsequent to long-term storage. This assessment and the resulting determination of a coefficient of variation for SPMDs will advance knowledge on precision

of these devices and have implications for interpretation of environmental concentrations measured therein.

The assessment of toxicity of mixtures in risk assessment: Old ideas in new light (PL)

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The assessment of the toxicity of mixtures of metals and organic compounds has been developed along multiple separate lines for almost a century by various scientific and medical disciplines including toxicology, industrial hygiene, and ecotoxicology. This has led to confusion over terminology and various approaches, but there has been progress toward an understanding of the assumptions and limitations of the various methods. This sheds new light on the value of some of the older ideas. The current state of the art in the various approaches to the assessment of the toxicity of mixtures will be reviewed and contrasted, including simple addition, toxic units and toxic equivalent methods. Examples will be provided.

The effect of dispersed mine tailings on sediment microbiomes (PO)

Susan Baldwin[1], Heath Garris[2], Jon Taylor[1] and Lauchlan Fraser[2]

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Many mines are surrounded by aquatic ecosystems. When assessing impacts from mining on these important environments, very small single-celled organisms are largely overlooked compared with multi-cellular ones, although they are just as critical for ecosystem health. Sediment microbiomes play important roles in carbon, nitrogen, sulfur and metal cycling that impact quality of the overlying water. Shifts in the sediment microbiome community composition might have implications for metal release or sequestration, depending on what functional groups are present. After the Mount Polley Tailings Storage Facility breach in August 2014, which dispersed tailings into Hazelton Creek, Polley Lake and Quesnel Lake, we measured the microbial community composition and metabolic potential of the impacted littoral zones. Heavily impacted areas had microbiomes very different from those found in non-impacted areas. While the microbial communities within different vertical layers of the non-impacted littoral zones were quite distinct, evidence of mixing was observed in samples from impacted sites. Additionally, certain species were enriched in the deposited tailings, such as *Thiobacillus denitrificans*, a species that couples oxidation of sulfur compounds with denitrification, which has implications for the sulfur cycle.

An evaluation of artifactual toxicity in fathead minnow tests caused by microbes (PO)

Madison Lehti[1], James Elphick[1], Gary Lawrence[2] and Nick Manklow[3]

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Periodic toxicity tests using a ~30-day exposure to fathead minnows (*Pimephales promelas*) using ambient water samples collected upstream and downstream of mine operations in British Columbia (BC) resulted in adverse responses in larval fish in a number of samples in a manner that did not appear to be attributable to chemical constituents in the samples. The effects were on survival and generally occurred between Days 6 and 12 of exposure; surviving fish showed no adverse sublethal effects on growth or

incidence of deformities. Toxicity Identification Evaluation efforts determined that naturally-occurring microbes, most likely fungi, were the cause of toxicity. Amendment of the samples with copper was proposed and trialed as a prophylactic method to inhibit microbial growth; treatment with a concentration of copper equivalent to the 30-day average BC water quality guideline for copper appears to be sufficient to remove the confounding influence of microbial growth.

The Freshwater Inventory and Surveillance of Mercury (FISHg) Network: Long-term mercury monitoring in water, sediment and fish in Canadian lakes (PO)

Melissa Gledhill[1], Alain Armellin[1], Sean Backus[1], Mandi Clark[1], Marlene Evans[1], Christine Garron[1], Michael Keir[1], Benoit Lalonde[1], Mark Sekela[1] and Jim Syrgiannis[1]

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In 2008, the Clean Air Regulatory Agenda (CARA) was established by the Government of Canada to regulate and monitor emissions from Canadian industrial, transportation and manufacturing sectors. Under CARA, the Mercury Science Program addressed emissions and deposition of mercury and its fate and potential effects in the Canadian landscape. The CARA Freshwater Inventory and Surveillance of Mercury (FISHg) network was established to determine mercury concentrations in predator and prey fish in lakes distributed across Canada and to track changes in those concentrations over time. A total of 19 lakes have been sampled since 2008. Of those lakes, a core set of six lakes has been monitored every year of the program. These six lakes are located in watersheds with little or no disturbance and no point source terrestrial-based mercury inputs. All sampling was conducted using standardized field protocols and laboratory analyses to enable comparability of data between lakes as well as between years. *In situ* depth profiles were conducted to identify thermal and oxygen stratification. Data collected for the fish were species, age, sex, length, weight, stable isotopes of carbon and nitrogen, and 30 metals including mercury in both fillet and whole fish tissues. Water samples were analyzed for nutrients, ions, physical parameters, metals, mercury, methylmercury and chlorophyll a. Sediment samples were analyzed for inorganic and organic carbon, organic nitrogen, sulfate, metals, mercury and methylmercury. Additionally, sediment cores were collected at five of the six long-term lakes and have been dated and analyzed for mercury and methylmercury. With nearly 10 years of monitoring data, temporal patterns and spatial comparisons between the lakes are presented.

Three-spined stickleback (*Gasterosteus aculeatus*) as a new Environment and Climate Change Canada (ECCC) vertebrate reference species for acute lethality testing of saline effluents discharged to marine environments (PO)

Ryan Hennessy[1], Leana Van der Vliet[1], Lisa Taylor[1], Paula Jackman[1] and Rick Scroggins[1]

[1]Environment and Climate Change Canada

As part of the 10-year review of the *Metal Mining Effluent Regulations* (MMER) and the development of mines in the Canadian far north, a need has been identified for new biological reference methods to measure acute lethality in saline effluent from mine operations discharging to the marine environment. A marine fish test method is needed for monitoring and compliance purposes as part of the amended MMER (tentatively scheduled for publication in *Canada Gazette*, Part II, by the end of March 2018) and would be a substitute for the rainbow trout (*Oncorhynchus mykiss*) test (EPS 1/RM/13) used for the assessment of acute lethality in freshwater mining effluents. To select an acceptable saline

effluent testing method, several fish species were considered, using criteria such as salinity tolerance, cold water suitability, the level of experience Canadian ecotoxicological laboratories have employing the species in routine testing, availability of biological suppliers, year-round availability of juveniles suitable for testing, ecological relevance to Canada (especially in northern waters), and representation in the literature—including method standardization work carried out by other organizations. Seventeen potential vertebrate species were explored using the aforementioned criteria. *Gasterosteus aculeatus* was selected from these seventeen species as the most appropriate for Environment and Climate Change Canada's acute lethality requirement under an amended MMER. The selection of three-spined stickleback was primarily influenced by the extensive method development and standardization work that has been performed with this species in addition to its meeting of all other criteria.

Acartia tonsa as a new Environment and Climate Change Canada (ECCC) invertebrate reference species for acute lethality testing of saline effluents discharged to marine environments (PO)

Ryan Hennessy[1], Leana Van der Vliet[1], Lisa Taylor[1] and Rick Scroggins[1]

[1]*Environment and Climate Change Canada*

A review of Canadian *Metal Mining Effluent Regulations* (MMER) has recently identified the need for a new monitoring and compliance test method. This new method is necessitated by a number of mining operations in the Canadian far north who have requested that they be allowed to discharge saline effluent from their operations to marine environments. Currently, the invertebrate Reference Method prescribed in the MMER for acute lethality testing of mining effluents uses *Daphnia magna* as the test species (EPS 1/RM/14). *D. magna*, while an excellent species for the testing of freshwater effluents, is inappropriate for the testing of saline effluents which are discharged to marine or brackish environments, as the species is intolerant of salt concentrations in excess of four parts per thousand. The MMER will be amended in the near future to prescribe acute lethality testing with marine fish and invertebrate species, so there is need for a marine invertebrate standardized test for monitoring and compliance purposes. To serve as an acceptable saline effluent testing method the invertebrate species must be: salt tolerant, capable of surviving in cold water, easily cultured in a laboratory setting, and found in Canadian marine and estuarine environments where it plays both top-down and bottom-up roles in the food web. These criteria are fulfilled to some degree by several marine invertebrate species but *Acartia tonsa* is set apart from all others by the extensive effluent quality control work that has been performed with it in other jurisdictions. Previous method standardization accomplished, and expertise developed, with *A. tonsa* by other agencies (e.g., the International Standards Organization and the Italian Ministry of the Environment and Territory and Sea Protection) can be leveraged to drastically reduce the time and cost necessary to develop an Environment and Climate Change Canada (ECCC) standard Reference Method. When taking the relatively tight timelines into account, *A. tonsa* is the only sensible marine species for ECCC to pursue for the acute lethality requirement under an amended MMER.

Minimizing confounding influences in fish health environmental effects monitoring data by effectively classifying gonad maturation stages (PO)

Melanie Jaeger[1], Collin Arens[1], Timothy Barrett[2] and Rainie Sharpe[1]

[1]Golder Associates Ltd., [2]Minnow Environmental

Effective environmental effects monitoring requires accurate fish reproductive stage classification to reduce unnecessary variability in fish health data. Data must be categorized by sex (i.e., male, female or unknown) and maturity (e.g., maturing, ripe, spent) prior to performing statistical analyses. Multiple gonad maturity classification systems exist and various terminologies are used among proponents, consultants, regulators, and laboratories, all of which complicates communications and effective comparisons among studies. Golder Associates Ltd. (Golder) has developed a gonad maturity classification system, based broadly on information presented by Brown-Peterson et al. (2011), which allows for a consistent and reproducible approach of gonad classification to be applied across multiple studies and regions. The gonad maturity classification system includes seven gonad maturation stages and five sub-stages (which account for the presence of parasites, which typically confound fish health analyses) and may be applied and understood by field staff during sampling programs and technical staff (i.e., histologists) during subsequent laboratory analyses. Macro and histological images of gonads from both large-bodied and small-bodied fish studied during the 2015 and 2016 field seasons will be presented. This poster will present specific examples of the gonad maturity classification system developed by Golder for fish species most relevant to environmental effects monitoring in northern Canada, with specific examples from the Northwest Territories.

Fish health in Alberta's Athabasca oil sands: Developing baseline to assess future change (PO)

Mark McMaster[1], Heather Keith[2], Gérald Tétreault[1], Jim Bennett[1], Marlene Evans[1], Thomas Clark[1], Richard Frank[1] and Joanne Parrott[1]

[1]Environment and Climate Change Canada, [2]Hatfield Consultants

As part of the Canada-Alberta Joint Oil Sands Monitoring (JOSM) program, fish health within the Athabasca River watershed is being evaluated using methods developed for the Canadian Environmental Effects Monitoring (EEM) program. Data will provide a baseline against which future changes in fish health will be judged and will be compared to historical studies, where possible, to assess change. On the Athabasca River, the white sucker (*Catostomus commersonii*) has been selected as a large-bodied sentinel species and the trout-perch (*Percopsis omiscomaycus*) as a small-bodied sentinel species. Walleye (*Sander vitreus*) are also being used for fish contaminant monitoring. Where possible, study designs require the collection of fish at sites off the oil sands deposit (reference), at sites within the natural deposit but upstream of development, and at sites downstream of development. We will discuss mainstem fish studies to date, with recommendations on how to proceed with the monitoring program post-2015.

General Toxicological Effects of Contaminants in Aquatic Species

Interactive effects of 2- and 3-way mixtures of copper, nickel and cadmium on rainbow trout (*Oncorhynchus mykiss*) olfaction (PL)

Sarah Bogart[1], Ebrahim Lari[1], Aditya Manek[1], William Dew[2] and Greg Pyle[1]

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Although single metals have been demonstrated to impair fish olfaction of food, mates, and predators, our understanding of the effects of metal mixtures on the olfactory system is limited. As such, we characterized the acute, 2- and 3-way metal mixtures effects of cadmium (Cd), nickel (Ni), and copper (Cu) on rainbow trout (*Oncorhynchus mykiss*) olfaction at their respective olfactory-based IC_{20} values. To construct olfactory-based impairment curves for IC_{20} calculations, fish were exposed to a geometric concentration series of single metals for 24 hours and then their olfactory acuity was measured via electro-olfactography (EOG). Resultant EOG-IC_{20} values for Cd and Cu were 24.4 $\mu g \cdot L^{-1}$ and 4.6 $\mu g \cdot L^{-1}$, respectively; however, no IC_{20} could be calculated for Ni because it did not impair olfactory function at environmentally relevant concentrations. Thus, instead of an IC_{20} value, the Alberta (Canada) acute water quality guideline (WQG) for Ni (770 $\mu g \cdot L^{-1}$) was used in all mixtures exposures containing Ni. A binary mixture of Cd and Cu produced a less-than-additive olfactory impairment, while all other mixtures (Cd Ni, Cu Ni, Cd Cu Ni) resulted in more than additive olfactory impairment after 24 hours of exposure. Interestingly, the EOG-IC_{20} value for Cu was 6-fold lower than the current Alberta acute WQG for Cu. Our results suggest that some of the criteria used for the protection of aquatic life, such as the acute Cu WQG, may not protect against adverse effects on the olfactory system of fish. Additionally, interactions between some contaminant mixtures, such as Ni with Cd or Cu, may increase the toxicity of the individual contaminants. We suggest that future WQG should consider the potential impact of metal mixtures interactions on fish olfaction.

Metabolic and cardiovascular effects of dietary selenomethionine exposure in adult zebrafish (*Danio rerio*) (PL)

Connor Pettem[1], David Janz[1] and Lynn Weber[1]

[1]University of Saskatchewan

Selenium (Se) is an essential micronutrient involved in important metabolic functions for all vertebrate species. As Se is reported to have a narrow margin between deficiency and toxicity, there is growing concern surrounding the adverse effects of elevated Se exposure caused by anthropogenic activities. Oviparous vertebrate species, especially fish, are highly susceptible to elevated dietary Se exposure. Recent studies have reported that elevated dietary exposure of fish to selenomethionine (Se-Met), the primary form of Se in the diet, can alter metabolic capacity, energy homeostasis, and swimming performance, and can cause a greater incidence of early life-stage deformities and mortality. This study aimed to further investigate mechanisms of Se-Met toxicity, particularly potential underlying cardiovascular implications of chronic exposure to environmentally relevant concentrations of dietary Se-Met in adult zebrafish (*Danio rerio*). Adult zebrafish were fed either control food or Se-Met spiked food (10.27 or 28.81 $\mu g\ Se \cdot g^{-1}$, dry weight) for 90 days at 5% body weight per day. Following exposure, high resolution B-mode and Doppler ultrasound was used to characterize cardiac and vascular function.

Chronic dietary exposure to Se-Met caused significant decreases in blood velocity through the atrioventricular (AV) valve, reduced stroke volume and cardiac output, and impaired ventricular diastolic filling. These effects may lead to further cardiovascular complications. Metabolic endpoints investigated included muscle glycogen and triglyceride stores, and fish fed with Se-Met spiked food had elevated energy stores, suggesting impaired energy homeostasis and metabolic dysfunction. Expression of mRNA genes of interest was quantified by use of Real-Time Quantitative Polymerase Chain Reaction (qRT-PCR) and current findings are being examined. The results of this study suggest that chronic exposure to dietary Se-Met can alter both cellular and physiological responses, and such consequences could threaten fitness and survivability of fish.

Natural selection and aquatic ecotoxicology in the oil sands: Has pre-exposure to natural sources of bituminous oil sands toxicants stimulated local adaptation in forage fish populations inhabiting Alberta's oil sands region? (PL)

Sylvia Chow[1] *and Greg Pyle*[1]

[1]*University of Lethbridge*

The lower Athabasca River basin of northern Alberta travels through the bituminous rich deposits of the McMurray geologic formation, and it is within this region where aquatic organisms are exposed to natural fluvial erosion of bitumen-rich oil sands. Extent and potentiality of natural selection through the scope of local adaptation of indigenous fish populations to natural and anthropogenic bituminous sources are not well known, but it is with this knowledge that environmental impact assessment of the industry may be improved upon. Following a reciprocal cross transplant between sites containing natural bitumen and downstream of industry, a 28-day chronic exposure experiment was conducted using a small forage fish, the fathead minnow (*Pimephales promelas*). Reference fish olfactory acuity in response to social cues such as conspecific alarm cues and taurocholic acid was impaired to levels that were similar to fish downstream of industry after having been transplanted to either of the bituminous sites. Similarly, respiration rates were observed to decrease to levels that were detected in fish that resided in bituminous toxicant-containing waters prior to experimentation. Fish from downstream and natural bitumen sites had lower olfactory acuity and respiration rates than fish from upstream site who did not exhibit any changes in the two endpoints before and after the chronic exposure. Fish without pre-existing contact with bitumen—natural or otherwise—displayed impairment in olfaction and respiration to levels comparable to fish from within the oil sands region. Biomarker for toxicant exposure increased in the downstream fish population that was transplanted into the natural bitumen-containing tributaries. Fish from populations inhabiting bitumen-rich environments did not exhibit local adaptation in the measured physiological traits as was previously hypothesized. Physiological scope of fish from natural bitumen and downstream-of-industry waters may be limited in comparison to reference fish without previous bitumen exposure, which supports the lower survival rates observed in the former. Data in this study illustrate how differing toxicant loads will affect the ability of a forage fish population to cope with particular environmental stressors. Although no evidence of local adaptation was observed, the differences in physiological responses indicate an effect of oil sands related toxicants on these fathead minnow populations. Understanding that these forage fish populations exhibit a limited physiological scope can inform and improve reclamation and remediation efforts within Alberta's oil sands region.

Assessment of acute and chronic toxicity of diluted bitumen (dilbit) and weathered dilbit to fishes and aquatic invertebrates (PL)

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In this study, funded by Fisheries and Oceans Canada's National Contaminants Advisory Group, the acute toxicity of diluted bitumen (dilbit) and weathered dilbit was assessed using two fish species, rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*), and an invertebrate species, *Daphnia magna*. Chronic toxicity was also evaluated on fathead minnow and an invertebrate species, *Ceriodaphnia dubia*. In tests carried out with rainbow trout, dilbit demonstrated a significantly higher toxicity (96-hour LC_{50} = 5.66 g·L^{-1}) compared to weathered dilbit (96-hour LC_{50} > 18 g·L^{-1}). For fathead minnow, dilbit also demonstrated a significantly higher toxicity (96-hour LC_{50} = 0.628 g·L^{-1}) compared to weathered dilbit (96-hour LC_{50} = 2.06 g·L^{-1}). Chronic tests showed that fathead minnow lethality toxicity was also higher for dilbit (7-day LC_{50} = 0.593 g·L^{-1}) compared to weathered dilbit (7-day LC_{50} = 1.31 g·L^{-1}), whereas larval growth toxicity was lower for dilbit (7-day IC_{25} = 0.312 g·L^{-1}) compared to weathered dilbit (7-day IC_{25} = 0.096 g·L^{-1}). No mortality was observed during exposure in *D. magna* exposed to the water-accommodated fraction (WAF) performed with dilbit-10 g·L^{-1}, but mortality was 27% in dilbit-32 g·L^{-1}. Lethal toxicity (LC_{50} = 6.43 g·L^{-1}) was observed in *C. dubia* exposed to WAF performed with dilbit while no mortality was observed with weathered dilbit. The reproductive effects of *C. dubia* are more important with dilbit (IC_{25} < 1.0) than with weathered dilbit (IC_{25} = 3.99 g·L^{-1}). Volatile organic compounds (VOCs) were present in significant concentrations in all different concentrations of WAF. VOC concentrations decreased significantly when the WAF is made from weathered dilbit compared to dilbit. This reduction in VOC concentrations found in the WAF carried out with weathered dilbit is probably due to the weathering of dilbit. Benzene and toluene are 91.7% to 93.8% VOC for WAF made from dilbit, and 73.9% and 85.6% VOC when WAF is made from weathered dilbit. Generally, VOCs decreased significantly over time during the various toxicological tests. Polycyclic aromatic hydrocarbons (PAHs) are not affected by the process of “weathering” since their concentrations are similar in both types of dilbit. As with VOCs, PAH concentrations decreased relatively quickly after 24 hours of exposure and similarly for the tests with *D. magna* and *C. dubia*. For trout assays under continuous aeration, PAH concentration decreases were even faster. Also, there seemed to be a saturation of VOCs and PAHs beyond 10 g·L^{-1} of dilbit. Total petroleum hydrocarbon (TPH) concentrations were very low in all WAF and, as with VOCs, weathered dilbit led to a decrease in TPH concentrations.

Identifying serum biomarkers of diluted bitumen exposure in sockeye salmon (PL)

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Diluted bitumen (dilbit) is a major crude oil product of oil sands extraction that is transported from Alberta, Canada, across North America by rail and pipeline. Several plans to increase pipeline infrastructure raise concerns about environmental contamination from dilbit spills, particularly in sensitive aquatic habitats like the coastal watersheds of British Columbia. Proposals to expand dilbit transport through this critical Pacific salmon habitat could impact culturally and economically important species like sockeye (*Oncorhynchus nerka*). For example, we recently showed that juvenile sockeye exposed to low, environmentally relevant concentrations of the water-soluble fraction of dilbit (WSFd) have impaired performance in a critical swimming speed test (U_{CRIT}). In this parallel study, we

characterized the serum proteome of juvenile sockeye exposed to 0 or 66.7 µg·L^{-1} total polycyclic aromatic hydrocarbons (PAHs) from WSFd for 1 and 4 weeks. Half of the exposed fish in each treatment were sampled at exhaustion from the U_{CRIT} test, while the other half were not exercised. Serum proteome analysis was conducted using isobaric tags for relative and absolute quantitation (iTRAQ) paired with mass spectrometry. The main goals of this study were to: (1) identify candidate biomarkers of WSFd exposure that could be developed into biomonitoring tools, and (2) determine if the serum proteome of WSFd-exposed fish reflects compromised swimming performance. Three proteins were robust predictors of WSFd exposure, independent of exposure duration or exercise: complement component C7, hemopexin, and α-2-marcoglobulin. In non-exercised salmon, WSFd altered serum levels of 17 proteins related to coagulation, immune, and stress responses. In WSFd-exposed and exercised salmon, 20 proteins were significantly altered, including increased abundances of contractile proteins, suggesting that WSFd exposure exacerbates exercise-induced muscle injury and may compromise the ability of salmon to sustain and recover from intense exercise. This study lays the groundwork for developing simple, non-lethal biomonitoring tools to assess salmon population health following dilbit exposure, and may be helpful in predicting if exposed fish can endure their exhausting seaward migrations. Supported by the National Contaminants Advisory Group of Fisheries and Oceans Canada.

Comparing the photo-induced toxicity of polycyclic aromatic hydrocarbons in two amphibian species, *Xenopus laevis* and *Lithobates sylvaticus* (PL)

Zachary Currie[1] *and Natacha Hogan*[1]

[1]*University of Saskatchewan*

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants derived primarily from the incomplete combustion of organic matter. PAHs are generally not acutely toxic to aquatic organisms; however, ecologically relevant intensities of ultraviolet (UV) light have been shown to increase the acute toxicity of certain PAHs. Early life-stages of amphibians may be particularly susceptible to PAH photo-induced toxicity as they are translucent, have permeable skin and undergo embryo and larval development in shallow ponds. Limited studies have investigated the potential photo-induced toxicity of PAHs in amphibian species and it is unknown whether the model organism *Xenopus laevis* is a good predictor of PAH photo-induced toxicity in native species. The objective of the present study was to evaluate and compare the sensitivity of *Xenopus* and an ecologically native species (wood frog, *Lithobates sylvaticus*) to the photo-enhanced toxicity of PAHs. Individual 96-hour tests were performed in which tadpoles were exposed to anthracene, naphthalene, acridine or benzo[a]pyrene (BaP) for 8 hours in the dark, transferred to clean water, and then exposed to 630 µW·cm^{-2} UVA (315-400nm) and 110 µW·cm^{-2} UVB (285-315nm) light for 12 hours. Results were compared using mortality, growth, body burden, and whole-body transcriptomic responses. Results showed that 200 µg·L^{-1} anthracene, 10 µg·L^{-1} BaP and 100 µg·L^{-1} BaP interacted synergistically with UV light to cause a significant increase in tadpole mortality. It was also found that 20 and 200 µg·L^{-1} anthracene interacted synergistically with UV light to cause a significant decrease in tadpole length. Acridine and naphthalene were not found to significantly affect tadpole mortality or growth at any concentrations tested. Based on mortality data, it appears that *Xenopus* is more sensitive to the photo-induced effects of PAHs compared to the wood frog. This may establish *Xenopus* as a protective model for native species of amphibians with regards to the photo-induced toxicity of PAHs. Overall, anthracene and BaP toxicity in larval amphibians is enhanced with exposure to ultraviolet radiation, while such photo-induced toxicity is not demonstrated by acridine or naphthalene.

Cardiac and metabolic effects of benzo[a]pyrene and 5-azacytidine in juvenile rainbow trout (*Oncorhynchus mykiss*) (PL)

Fred Leal[1]

[1]*University of Saskatchewan*

Benzo[a]pyrene (BaP) is a ubiquitous environmental contaminant that is rapidly metabolized but has been reported to induce persistent cardiac and metabolic effects in fish. Juvenile rainbow trout (*Oncorhynchus mykiss*; n=20) were injected once daily for 2 consecutive days with corn oil (control vehicle) or BaP (0.1 and 1 $mg \cdot kg^{-1}$). On Days 4 and 7 (n=10/group/day), fish underwent cardiac ultrasound, followed by euthanasia, dissection, and collection of arterial blood, heart, liver, and red muscle for analysis of BaP residues in liver, heart, and muscle (through high-performance liquid chromatography (HPLC)); analysis of tissue glycogen and triglycerides (TG) content; liver and muscle enzyme activities of lipoprotein lipase (LPL; hydrolyzation of TG into fatty acids and their transport into cells), 3-Hydroxyacyl-CoA dehydrogenase (HOAD; marker of beta-oxidation of fatty acids), citrate synthase (CS; marker of aerobic metabolism), lactate dehydrogenase (LDH; marker of anerobic metabolism), and L-lactate level in blood, liver, and muscle (secondary marker of anaerobic metabolism). Analysis of BaP residues in tissues showed a severe reduction of BaP from Day 4 to 7 in the liver (205.81±65.08 to 4.17±1.32 $\mu g \cdot kg^{-1}$ dry mass; mean ± standard error of mean) and in the muscle (62.64±19.8 to 1.78±0.594 $\mu g \cdot kg^{-1}$ dry mass; mean± standard error of mean) in the high BaP treatment group. However, in the heart, levels were found to be below detection levels for both days. Cardiac effects were noticed on Day 4 when there were decreases in peak and mean velocities of blood flow through the atrioventricular valve (consistent with increased ventricular wall stiffness) as well as duration of blood flow through the ventriculobulbar valve and into the aorta. All cardiac function parameters returned to control values by Day 7. In the liver, LPL and HOAD activities remained elevated at Day 7 in both the high and low BaP treatments, while glycogen stores were decreased compared to control for both BaP groups. In the muscle, glycogen stores were also persistently decreased at Day 7 in both BaP treatment groups. Taken together, most cardiac effects were transient and correlated with BaP residue in the body, but not the heart. In contrast, trout exposed to BaP underwent a shift toward more glycolytic metabolism that persisted at 7 days after exposure, a time when BaP body burden had almost disappeared. Greater reliance on glycogen as an energy source may threaten the survival of wild *Oncorhynchus mykiss* since utilization of TG is more metabolically advantageous for the long-distance swimming these fish perform during migration.

Determination of acute and sub-chronic toxicity of emerging contaminants in early life-stages of three Canadian fish species (PL)

Dayna Schultz[1], *Song Tang*[1], *Christie Miller*[1], *Steve Wiseman*[1], *David Janz*[1] *and Markus Hecker*[1]

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In recent years, emerging contaminants have gained notoriety due to their ubiquity in the aquatic environment as well as the lack of data available regarding their toxicity to wildlife and humans. Emerging chemicals (ECs) of concern, such as hexabromocyclododecane (HBCD), silver nanoparticles (AgNPs), short-chain chlorinated paraffins (SCCP), 17α-ethynylestradiol (EE2) and Prozac® (FLX) primarily enter the aquatic environment as mixtures through municipal wastewater effluent (MWWE). MWWE, which is typically a mixture of industrial, commercial, and household wastes, may be released into receiving waters with little to no treatment, which is not uncommon, especially in rural Canadian

municipalities. Most data to date have been garnered using standard laboratory species, which may not be particularly relevant to northern species, considering the potential role of life history, trophic level, physiology, and climate on the species-specific toxicity of chemicals. Consequently, inaccurate extrapolation from standard laboratory species to species native to northern ecosystems is a cause for concern and represents a significant uncertainty factor in ecological risk assessment. In this study, rainbow trout (*Oncorhynchus mykiss*), lake trout (*Salvelinus namaycush*), and northern pike (*Esox lucius*) gametes were exposed to six waterborne concentrations of EE2, FLX, MWWE, and AgNPs, where the lowest doses were selected based on environmental relevance and increased incrementally thereafter. Exposures were continuous flow-through and subsamples were collected at critical developmental stages to assess acute and sub-chronic toxicity of all test chemicals. Initial findings suggest that these three species vary significantly in their sensitivities towards the aforementioned ECs. Ongoing work aims to fully elucidate biochemical and histological anomalies associated with exposures to the six ECs and focuses on characterizing the effects of these ECs on native fish species in comparison to one another as well as standard laboratory fish models. Overall, this work will aid in the development of more appropriate environmental risk assessment strategies for native fishes to ECs of concern. Supported by the National Contaminants Advisory Group of Fisheries and Oceans Canada.

Assessing the toxicity and bioaccumulation of substituted phenylamine antioxidants in sediment exposures with *Hyalella azteca* and *Tubifex tubifex* (PL)

Adrienne Bartlett[1], Ryan Prosser[1], Heather Ikert[1], Daniel Schissler[1], Danielle Milani[1], Patricia Gillis[1], Joanne Parrott[1] and Vimal Balakrishnan[1]

[1]*Environment and Climate Change Canada*

Canada's Chemicals Management Plan (CMP) was announced in 2006 to regulate chemicals detrimental to human health and the environment. Few toxicological data are associated with the numerous chemicals used in industrial processes that are listed in the CMP. Substituted phenylamine antioxidants (SPAs) are produced in high volumes and have a range of applications, including dyes, photosensitizers, lubricants, dispersants, and adhesives. They were identified by the CMP as requiring further study due to their likely environmental persistence, potential for bioaccumulation, and inherent toxicity to aquatic organisms. We selected four SPAs for examination based on the range of log K_{OW} values: diphenylamine (SPA 2), N-phenyl-1-naphthylamine (SPA 6), N-(1,3-dimethylbutyl)-N'-phenyl-1,4-phenylenediamine (SPA 8), and 4,4'-methylene-bis[N-sec-butylaniline] (SPA 12). Our objective was to assess the toxicity and bioaccumulation of these four SPAs in sediment exposures with two species of benthic invertebrates: *Hyalella azteca* (amphipod) and *Tubifex tubifex* (oligochaete). Four-week spiked sediment exposures were conducted with each SPA, and effects on survival (both species), growth (*H. azteca*), and reproduction (i.e., production of cocoons and juveniles; *T. tubifex*) were assessed. Additional exposures were conducted to determine bioaccumulation of SPAs in *T. tubifex*. Chemical analysis of water, sediment, and tissue is ongoing; results are currently based on nominal concentrations ($\mu g \cdot g^{-1}$ dry weight sediment). Survival of *H. azteca* was reduced by SPA 2, 6, and 8, with 28-day LC_{50}s ranging from 130 $\mu g \cdot g^{-1}$ (SPA 2) to 1300 $\mu g \cdot g^{-1}$ (SPA 6), but was not affected by SPA 12 at any concentration tested (up to 2000 $\mu g \cdot g^{-1}$). Growth of *H. azteca* was a less sensitive endpoint than survival, and was not reduced by any concentration tested (up to 200, 1200, 1000, and 2000 $\mu g \cdot g^{-1}$ for SPAs 2, 6, 8, and 12, respectively). Survival of *T. tubifex* was reduced by all SPAs, with 28-day LC_{50}s ranging from 220 $\mu g \cdot g^{-1}$ (SPA 2) to 1800 $\mu g \cdot g^{-1}$ (SPA 12). Reproduction of *T. tubifex* was a more sensitive endpoint than survival: four-week EC50s for production of juveniles were 3-9 times lower than corresponding 28-day LC_{50}s. SPA 2 was the most toxic to the survival of both species, and SPAs 2 and 8 were the most toxic to the reproduction of *T.*

tubifex. However, these results are based on nominal sediment concentrations; therefore, the relative toxicity of the SPAs, as well as the concentration ranges causing toxicity, may change once measured exposure concentrations are available. In addition, the calculation of biota-sediment bioaccumulation factors will provide information on the bioavailability of SPAs to *T. tubifex*. The results of this study will be compared to measured concentrations of SPAs in biosolids and wastewaters from municipal wastewater treatment facilities in Canada, and will support environmental risk assessments to determine if SPAs could impact freshwater organisms.

The effect of sediment-derived substituted phenylamine antioxidants to different life-stages of a freshwater mussel (PL)

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Substituted phenylamine antioxidants (SPAs) are produced in relatively large volumes and incorporated into a variety of consumer products (e.g., polymers, lubricants, dyes, and adhesives). Based on their physical and chemical properties, SPAs could be persistent, bioaccumulative and/or toxic; consequently, the Government of Canada is tracking this class of compounds under the Chemicals Management Plan to determine whether management activities may be warranted at a future time. The current study assessed the toxicity of four SPAs chosen from a list of twelve (diphenylamine (SPA 2), N-phenyl-1-naphthylamine (SPA 6), N-(1,3-dimethylbutyl)-N'-phenyl-1,4-phenylenediamine (SPA 8), and 4,4'-methylene-bis[N-sec-butylaniline] (SPA 12)) to three life-stages of the freshwater mussel *Lampsilis siliquoidea*. Acute sensitivity was determined by exposing the larval stage of mussels (glochidia) to SPAs for 48 hours in water. A negative relationship between the toxicity of SPAs and their solubility in water was observed. The 48-hour EC_{50}s for glochidia viability using measured concentrations were 5951, 606, 439, and 258 $\mu g \cdot L^{-1}$ for SPA 2, 6, 8, and 10, respectively. Juvenile (~1 cm in length) and adult *Lampsilis siliquoidea* were exposed to sediments spiked with individual SPAs for 28 days in aerated (static) vessels with a 3.5:1 water-to-sediment ratio. Mortality was assessed along with rate of feeding of juvenile mussels. In addition, reactive oxygen species, total glutathione, and lipid peroxidation in gill, digestive gland, and gonad of SPA-exposed adult mussels were measured to determine whether exposure to SPAs caused oxidative stress; bioaccumulation was also assessed in these tissues. Preliminary data analysis based on nominal concentrations showed that LC/EC_{50} values for the juvenile and adult mussels were >100 $\mu g \cdot g^{-1}$ sediment dry weight for all four SPAs examined. Analysis of tissue and sediment is ongoing; calculation of biota-sediment accumulation factors will provide information on the bioavailability of SPAs to freshwater mussels. The results of this study will support environmental risk assessment activities to determine if SPAs could impact freshwater ecosystems.

Reproductive and general health assessment of fathead minnow (*Pimephales promelas*) populations inhabiting an effluent-dominated stream, Wascana Creek, Saskatchewan (PL)

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), to resident wildlife as conventional wastewater treatment technologies are often incomplete or inefficient at removing such compounds. Waterbodies in the southern Canadian Prairies may be at particular risk of exposure to EDCs due to the uniqueness of prairie surface water systems. For example, during low flow periods, Wascana Creek, a small stream in southern Saskatchewan, can consist of over 90% treated effluent originating from the City of Regina's outdated lagoon-based treatment facility. The aim of this study was to characterize the potential endocrine-disrupting effects of municipal wastewater effluents on wild fathead minnow (*Pimephales promelas*; FHM) populations in an effluent dominated stream, Wascana Creek, Saskatchewan. Field studies were conducted on spawning FHMs (2014 and 2015) to assess responses in terms of overall health (condition factor, somatic indices), reproduction (secondary sexual characteristics, sex steroids in blood plasma, gonad histopathology, gene expression), and sex ratios. Fish collected downstream of the effluent fallout had lower gonadosomatic indices and significantly greater hepatosomatic indices compared to fish from upstream populations. There was significant disruption of regulation of key genes along the hypothalamus-pituitary-gonad-liver axis that are associated with reproductive processes. Additionally, in both male and female FHMs, gonadal degradation and delayed maturation was observed histologically. Exposed males displayed lower scores of secondary sexual characteristics. This case study highlights the potential ecological risks of EDCs associated with MWWEs and the need for implementing more effective and affordable measures to remove them at wastewater treatment plants.

Evaluation of an ozonation wastewater treatment technology for reducing the toxicity of micropollutants to fish (PL)

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Pharmaceuticals and personal care products (PPCPs) and endocrine-disrupting compounds (EDCs) are contaminants of emerging concern (CECs) due to their widespread detection in the aquatic environment and for their potential to induce biological responses in aquatic organisms at low, environmentally relevant concentrations. Since discharges of domestic wastewater are a major point source for the release of these micropollutants into surface waters, advanced treatment technologies are needed that can efficiently remove these compounds from wastewater. In this study, conducted in partnership with colleagues at McGill University and a private sector partner, Air Liquide, the toxic effects to fish of micropollutants extracted from ozonated and non-ozonated municipal wastewater effluent (MWWE) were measured in order to assess the effectiveness of ozonation in reducing toxicity. Juvenile rainbow trout (*Oncorhynchus mykiss*) were intraperitoneally injected with a mixture of five CECs commonly found in MWWE, and ozonated and non-ozonated wastewater extracts spiked with the five

model compounds. Toxicity testing was also completed using early life-stages of Japanese medaka (*Oryzias latipes*) exposed to the five model CECs, and to extracts prepared from ozonated and non-ozonated wastewater spiked with the model compounds. In juvenile trout, induction in plasma vitellogenin (VTG) and changes in levels of plasma steroids were observed in fish exposed to model CECs, in addition to elevated hepatic total glutathione (tGSH) levels, indicating that CEC mixtures have the potential to cause endocrine disruption and oxidative stress, respectively. Significant developmental toxicity was observed in medaka embryos exposed to all model CECs. In juvenile trout injected with extracts prepared from wastewater, ozonation significantly reduced induction of VTG, consistent with reductions in the measured concentrations of estrogenic contaminants. However, ozonation also significantly reduced hepatic tGSH levels and increased the hepatic GSSG-to-tGSH ratio, both of which are indicators of oxidative stress. Other *in vivo* biomarkers measured in trout showed no significant changes. Exposure of medaka embryos to extracts prepared from both non-ozonated and ozonated MWWE resulted in developmental toxicity that was significantly greater than the control group. These results indicate that the ozonation technology reduces the estrogenicity of wastewater, but treatment may induce oxidative stress and embryonic developmental toxicity due to the production of toxic by-products. These techniques will be used to assess the potential for biological effects in wastewater treated using a novel ozonation technology.

Targeted metabolomics for the monitoring of effects of pollution on sentinel species in the Great Lakes (PL)

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The Great Lakes in southern Ontario are affected by pollutants from a number of sources including wastewater treatment plants (WWTPs), industry and more. Pollutant monitoring over the years has detected pharmaceuticals and personal care products, metals, dioxins, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), pesticides and more in the water and associated sediment. Evaluating the effectiveness of regulation/policy changes on the health of the ecosystem is challenged by the number and chemical diversity of the pollutants. Multi-omic (transcriptomic, proteomic, and metabolomic) measurements of sentinel species that have been exposed to environmental samples from affected sites have the potential to evaluate early biological impacts in a controlled laboratory setting and to correlate these biological effects with measured contaminant levels. Such data can provide valuable insight into prioritizing and refining future contaminant measurement strategies, and can inform regulatory approaches earlier than toxicological tests focused on apical end-points. In this set of studies, rainbow trout (*Oncorhynchus mykiss*) and *Hexagenia* spp. were exposed to surface water, effluent and sediment from sites in Humber Bay and Toronto Harbour. Exposures were performed in a laboratory setting with each exposure lasting 48 hours. Concentration values of 219 metabolites, including amino acids, biogenic amines, ∑hexose, fatty acids, bile acids, acylcarnitines, sphingomyelins, and glycerophospholipids, were measured in rainbow trout liver and whole *Hexagenia*. In addition, concentrations of polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), metals, and other environmental contaminants were measured in the effluent, surface water and sediment samples. ANOVA, PCA, PLS-DA and OPLS-DA, were used to identify metabolites varying between the different location/concentration groups and to correlate metabolite changes with available water quality and contaminant concentration data. Preliminary analysis indicates that metabolomic differences in rainbow trout and *Hexagenia* spp. exposed to control samples compared with those exposed to both

surface water and effluent are modest. However, *Hexagenia* spp. sediment exposures show distinct metabolomic differences after exposure to field samples versus exposure to clean sediment with some indication of distance from WWTP gradient effects. This study is part of a larger two-year project evaluating the role of transcriptomic, proteomic and metabolomic effects measurement in understanding Great Lakes ecosystem health.

Relative sensitivity of the Pacific kelp (*Macrocystis pyrifera*) test and other bioassays used to evaluate adverse effects in marine ecosystems (PL)

Curtis Eickhoff[1], Emma Marus[1] and James Elphick[1]

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Effluents and wastewaters discharged into estuarine or marine environments are evaluated using a standard set of acute and sublethal bioassays for examining the potential for environmental effects. The sublethal bioassay using Pacific kelp, *Macrocystis pyrifera,* performed according to U.S. Environmental Protection Agency (USEPA) method EPA/600/R-95-136, is commonly used in the United States in conjunction with the echinoderm fertilization test, topsmelt growth and survival test, bivalve larval development test, and others. However, this bioassay is not as commonly utilized in Canada. In reviewing data sets involving two or more of these sublethal bioassays, it was apparent that the *Macrocystis* test often provided a sensitive endpoint comparable with other bioassays such as the echinoderm fertilization or bivalve larval development tests. This presentation compares the relative sensitivity of the kelp bioassay with a number of bioassays used for evaluating effects of effluents or sediments to estuarine and marine species. Additionally, we demonstrate that the kelp bioassay is sensitive to metals and insensitive to ammonia, making it an ideal bioassay for use in environmental effects monitoring programs in Canada for freshwater or saline discharges to marine environments. The insensitivity to ammonia also makes this test a strong alternative to sediment tests that are often confounded by ammonia toxicity when evaluating toxicity for dredge disposal or ocean disposal programs. This information is important for regulators and consultants when selecting bioassays for evaluating the hazard potential of wastewaters, surface waters, and/or sediments associated with industrial operations or contaminated sites.

Characterization of molecular toxicity pathways of selected emerging contaminants to elucidate species-specific sensitivity of three North American fishes (PO)

Alper James Alcaraz[1], Bryanna Eisner[1], Dayna Schultz[1], Song Tang[1], Steve Wiseman[1], Paul Jones[1], John Giesy[1] and Markus Hecker[1]

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Emerging chemical contaminants (ECC) are ubiquitous in the environment, and have become of increasing toxicological concern to humans and wildlife. ECCs include pharmaceuticals and personal care products, brominated flame retardants, and nanomaterials, among others, which are commonly discharged into surface waters through municipal wastewater and other sources as a result of human activities. However, little is known about the toxicological significance of most ECCs in the aquatic environment. Particularly, little is known about the effects of ECCs to fishes that are of commercial, cultural, and recreational relevance to North America and that are at risk of exposure with ECCs. Therefore, this study aims to investigate the responses and the underlying mechanisms of the exposure

to ECCs of three species of commercial, cultural, and recreational relevance, including lake trout (*Salvelinus namaycush*), white sturgeon (*Acipenser transmontanus*), and rainbow trout (*Oncorhynchus mykiss*). Specifically, the objectives of this study are to (1) characterize critical molecular toxicity initiating events (MIEs) associated with each ECC; (2) assess the conservation of gene expression signatures across fish species; and (3) construct ECC-induced molecular toxicity pathways, and anchor these to apical outcomes. Fishes at early life-stages will be exposed to three representative ECCs, namely nanosilver (AgNP), 17α-ethinylestradiol (EE2), or fluoxetine (Prozac®). Whole transcriptomic and proteomic coupled with receptor-binding, cellular and biochemical assays will be used to characterize critical toxicity pathways. Finally, mechanistic information from molecular toxicity pathways will be linked with apical responses across higher tier biological organizations. Linking results from open omics analyses with effects at higher levels of biological organization aims to identify and establish relevant biomarkers for environmental risk assessment. Supported by the National Contaminants Advisory Group of Fisheries and Oceans Canada.

Early-life exposure to diluted bitumen has lasting effects on brain morphology in sockeye salmon (PO)

Sarah Alderman[1], Feng Lin[2], Chris Kennedy[2], Anthony Farrell[3] and Todd Gillis[1]

[1]University of Guelph, [2]Simon Fraser University, [3]University of British Columbia

Diluted bitumen (dilbit), the crude oil product of oil sands extraction, is transported across North America by rail and pipeline. In British Columbia, Canada, proposals to expand dilbit transport to the Pacific coastline raise concerns about the ecological impacts of spilled dilbit, particularly in the aquatic environment. Sockeye salmon (*Oncorhynchus nerka*) are a culturally and economically important species at risk of dilbit exposure if these pipeline proposals are realized, but little is known about the specific toxicity of dilbit to fish, or the sensitivity of sockeye to the contaminants in dilbit. Developing fish are particularly sensitive to contaminants in crude oil, but the latent effects in the brain are not well understood. In the present study, sockeye salmon were exposed throughout early development (fertilization to swim-up) to four concentrations of the water-soluble fraction of dilbit (initial total polycyclic aromatic hydrocarbon (PAH) concentrations were 0, 13.7, 34.7 and 124.5 $\mu g \cdot L^{-1}$). At the end of the exposure period, alevin cyp1a mRNA levels were significantly elevated in all treatments relative to controls, confirming PAH bioactivity during the exposure. Phenotypically normal alevins were then transferred to clean water and raised for 9 months. While there were no differences in mass, length, or condition factor of juvenile salmon after 9 months, significant differences in regional brain volumes were apparent. Specifically, the olfactory bulbs, telencephalon, optic tectum, and cerebellum were all larger in fish exposed to 13.7 $\mu g \cdot L^{-1}$ total PAHs relative to controls. While the mechanisms of this response are not known, endocrine disruption during critical periods of neural development is a prime hypothesis. This study shows that fish exposed to low, environmentally relevant levels of the soluble-fraction of dilbit can have long-term effects on brain morphology. This is important for understanding the impact of dilbit contamination in salmon habitats. Supported by the National Contaminants Advisory Group of Fisheries and Oceans Canada.

The effects of diluted bitumen on the reproductive axis of the marine teleost, kelp greenling (*Hexagrammos decagrammus*) (PO)

Chris Kennedy[1]

[1]*Simon Fraser University*

Canada produces hundreds of thousands of cubic metres of bitumen per day, mostly from oil sands in northern Alberta. In British Columbia, existing and proposed pipelines are to carry diluted bitumen (dilbit) for transfer to ships destined for overseas markets, highlighting the potential risk of a spill of dilbit into the marine environment. Few studies exist on the toxicity of dilbit alone or in combination with common dispersants to any marine organisms. Successful reproduction in teleosts depends on a complex and highly regulated interplay between the pharmacokinetics and pharmacodynamics of naturally circulating sex steroids. Increasing evidence shows that environmental chemicals such as polycyclic aromatic hydrocarbons (PAHs) can impact the endocrine systems of vertebrates, mimicking or obstructing endocrine function and affecting normal biological function in a wide-ranging manner. The effects of a waterborne exposure to the dissolved fraction of diluted bitumen alone or in conjunction with the dispersant Corexit® 9500A on the kinetics of circulating levels of estradiol (E2) and testosterone (T) in mature male and female kelp greenling (*Hexagrammos decagrammus*) in pre-spawning and spawning condition were investigated. Plasma E2 and T concentrations were reduced significantly in both male and female greenling exposed to water containing bitumen or the bitumen/dispersant mixture; however, there were no significant effects due to the dispersant alone. As well as examining the effects of bitumen exposure on the early stages of spawning, the final stages were examined through the administration of the GnRH analogue des-Gly10[D-Ala6]LH-RH-ethylamide (which artificially promotes oocyte development). GnRH induced spawning steroid profiles as increased plasma E2 concentrations in control females, but not in bitumen-exposed fish. Detailed measurements of the time course of injected E2 and excretion into the bile followed by pharmacokinetic modelling techniques were used to aid in identifying the potential mechanism of endocrine disruption caused by bitumen exposure. The mechanism underlying reductions in sex steroids in pre-spawning and spawning salmonids appears to be unrelated to the induction of P450 (significantly induced in bitumen-exposed fish) and related biotransformation enzymes by bitumen. Induced biotransformation enzyme activities did not result in altered [3H]estradiol pharmacokinetics (e.g., terminal half-life) or total elimination of steroid in bile, suggesting that components of bitumen alter plasma E2 and T concentrations by other endocrine-disrupting mechanisms in an anti-estrogenic manner. This research highlights the potential reproductive effects that components of dilbit may have on marine teleosts at low $\mu g \cdot L^{-1}$ concentrations that would occur following a spill into the marine environment. Supported by the National Contaminants Advisory Group of Fisheries and Oceans Canada.

The lethal and sublethal effects of diluted bitumen and dispersants on Pacific marine algae, crustaceans, and teleost species (PO)

Chris Kennedy[1] *and Ranah Chavosi*[1]

[1]*Simon Fraser University*

Existing and proposed pipelines are to carry diluted bitumen (dilbit) from Canada's Alberta oil sands for transfer to ships destined for overseas markets, highlighting the potential risk of a spill of dilbit into the marine environment. Few studies exist on the toxicity of dilbit alone or in combination with dispersants to any marine organisms. When kelp (*Macrocystis pyrifera*) fronds were exposed to several

concentrations of the dissolved fraction of dilbit, no effects on fertilization or the germination of kelp zygotes were seen; however, dilbit exposure reduced growth significantly in a concentration-dependent manner. Adult spot prawns (*Pandalus platyceros*) that were not exposed to dilbit or dispersed dilbit were acclimated in a shuttle box apparatus in a choice/avoidance assay. Prawns were actively attracted to the dissolved fraction of dilbit at low concentrations but as concentrations increased, attraction declined. At all dilutions, prawns actively avoided dispersed dilbit. Pink salmon swim-up fry in fresh water and 3-g fry in seawater that were not exposed to dilbit were acclimated in a shuttle box apparatus, in a choice/avoidance assay. Pink salmon swim-up fry in fresh water did not actively avoid dilbit at any concentration. Larger fish avoided dilbit in seawater at concentrations as low as 40 $\mu g \cdot L^{-1}$. Pink swim-up fry and larger fry in seawater all actively avoided dispersed dilbit at most dilutions. Pink salmon fry were exposed to the dissolved fraction of dilbit and dispersed dilbit under various levels of hypoxia. The dissolved fraction of dilbit alone was not acutely toxic to pink salmon in seawater, however, dispersed dilbit resulted in mortality at higher concentrations. Hypoxia in conjunction with either dilbit alone or dispersed dilbit increased the toxicity of both of these mixtures in pink salmon. This research highlights the potential reproductive effects that components of dilbit may have on marine teleosts at low $\mu g \cdot L^{-1}$ concentrations that would occur following a spill into the marine environment. Supported by the National Contaminants Advisory Group of Fisheries and Oceans Canada.

Sublethal effects of diluted bitumen on Pacific sockeye salmon (*Oncorhynchus nerka*) early life-stages (PO)

Chris Kennedy[1] *and Feng Lin*[1]

[1]*Simon Fraser University*

Canadian pipeline companies have proposed a number of major new transmission pipelines that will transport diluted bitumen (dilbit) from the oil sands in northern Alberta to the coast of British Columbia (BC) for processing and for export to overseas markets. In BC, the routes of existing and proposed pipelines traverse the Fraser River Watershed, spawning habitat for multiple species of Pacific salmon. Similarly, in eastern Canada the proposed route of the TransCanada pipeline to Saint John, New Brunswick, traverses the St. Lawrence River as well as some 100 other Atlantic salmon-bearing rivers and watersheds. Leaks and ruptures to these pipelines will pose serious challenges to aquatic biota, including salmon. The effects of a waterborne exposure to the dissolved fraction of dilbit on various aspects of sockeye early life-stage physiology and behaviour were examined. Exposure of sockeye at the swim-up fry and juvenile stages resulted in concentration-dependent increases in oxygen consumption. No significant increases in oxygen consumption over baseline values occurred in yolk sac fry at any concentration, indicating that later-stage salmon may be experiencing a stress response and increased energetic costs associated with exposure. Sockeye fry continuously exposed to 4 concentrations of dilbit were fed *ad libitum* 3 times per day; dilbit exposure caused juveniles at two higher-treatment groups to gain significantly less mass than control fish. Fry and juveniles not exposed to dilbit were acclimated in a shuttle box apparatus and tested in a choice/avoidance assay to dilbit. Fish actively avoided bitumen, but this avoidance was strictly size-dependent, with a higher proportion of larger fish actively avoiding dilbit. Fry exposed to four concentrations of the dissolved fraction of dilbit for 0 hours, 24 hours, 4 days or 28 days were then acclimated in a shuttle box apparatus for a choice/avoidance assay to determine their ability to detect food. Unexposed fish responded to food extract; however, longer exposures to higher concentrations of dilbit resulted in the loss of attraction to food. These results show that exposure of sockeye salmon early life-stages results in several different sublethal toxicities, possibly reflecting a generalized stress response, as well as constituent-specific toxic mechanisms of action. These results will

aid in the development of risk assessment plans for managing salmon populations in the event of potential pipeline failures. Supported by the National Contaminants Advisory Group of Fisheries and Oceans Canada.

Risk of methylmercury (bio)availability in freshwater lakes (PO)

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Methylmercury (MeHg) toxicity is of particular interest in remote Canadian environments far from point sources of contamination, where high levels of MeHg are accumulating in top predators. The ecosystem variables that control this sensitivity of food webs are not well known. MeHg concentration in water and uptake into the base of the food web is one key factor controlling mercury entry into food webs. Few studies have directly considered photodemethylation reactions in combination with physical attributes of aquatic ecosystems to predict where and when dissolved MeHg may be available. To address this research gap we have used numerous controlled and semi-controlled experiments that focused primarily on the quantification of the relationships between solar radiation exposures, dissolved organic matter (DOM), and MeHg within six freshwater lake systems in Kejimkujik National Park and National Historic Site in south-western Nova Scotia. To better quantify the photodemethylation potential within these lakes we must determine: (1) the behaviour of photoreactive compounds, and (2) the availability of solar radiation with depth in water columns. Experimental treatments were 1-week long in summer and fall and were exposed to natural solar radiation over that time period. Photodemethylation rates were strongly controlled by DOM concentration (R^2=0.76) and were inversely related to rates of DOM phototransformation, which includes photomineralization (r=-0.66) and photobleaching (r=-0.83). Using these experimental outcomes, coupled with field measurements of solar radiation availability within lake water columns, we have developed a model for predicting photodemethylation potential and efficiency within the top 1 m^3 in oligotrophic dystrophic temperate lakes. These predictive results were then scaled up and used to calculate the overall photodemethylation potential in each of our six study lakes for comparison with MeHg concentrations in the corresponding food webs. This model may be appropriate for other aquatic ecosystems by simple standardization techniques depending on water quality characteristics such as DOM photoreactivity (structure), pH, and dissolved ionic species. Overall, this body of work yielded a method for predicting mercury availability to food webs depending on environmental and physicochemical factors. Climate change in temperate and boreal regions of Atlantic Canada is projected to increase rainfall amounts and occurrences and thus lead to browning of fresh waters and further inhibition to the photodemethylation pathway of MeHg reduction.

Validation of *in ovo* embryo microinjections using selenomethionine to simulate maternal transfer in the fathead minnow (*Pimephales promelas*) (PO)

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Selenium (Se) is a developmental toxicant of increasing concern because it can be released into the aquatic environment in significant amounts from natural and industrial processes. Inorganic Se released into surface water is biotransformed and bioaccumulated by microorganisms and algae as selenomethionine (SeM). SeM is then transferred via dietary means to higher trophic levels and

bioconcentrates in aquatic invertebrates and fish. Early life-stages of fish are highly sensitive to SeM exposure and are primarily exposed via maternal transfer. Developmental deformities (e.g., spinal curvature, craniofacial, edema) might occur as a result of embryo exposure to maternally transferred SeM. However, maternal transfer is difficult to study in native species of concern in Canadian ecosystems, especially for those species that are long-lived or endangered. This study will be the first step in developing an embryo injection model to help interpret maternal transfer of SeM. The fathead minnow (*Pimephales promelas*) was selected as a model organism based on its vast distribution throughout North America and extensive use in previous regulatory testing and research. Fathead minnow embryos will be injected with graded concentrations of SeM (5, 15, or 45 µg $Se \cdot g^{-1}$ dry mass embryo) and developmental endpoints will be compared with those from a parallel maternal transfer study. Establishing an embryo injection model for predicting toxicity of SeM through maternal transfer will make testing a broader range of species more feasible. Future research using this model will aim to determine Se sensitivity in early life-stages for native species of concern (e.g., white sturgeon (*Acipenser transmontanus*) and rainbow trout (*Oncorhynchus mykiss*)).

DFO's National Contaminants Advisory Group: Updates on ongoing biological effects of contaminants research (PO)

Cecilia Lougheed[1], *Cory Dubetz*[1], *Judith Leblanc*[1], *Alexander Okonski*[1]

[1]*Fisheries and Oceans Canada*

As a science-based federal government department, Fisheries and Oceans Canada (DFO) requires scientific evidence to facilitate the sound management of Canada's fisheries, and to advance sustainable aquatic ecosystems while fostering economic prosperity across maritime sectors and fisheries. The National Contaminants Advisory Group (NCAG) provides scientific information and advice to DFO on priority issues related to the biological effects of contaminants on aquatic ecosystems. The main functions of the group are to facilitate research projects through external researchers, to synthesize results and to develop science advice in support of DFO decision-making. Current priority research themes are: (1) oil and gas, (2) pesticides, (3) aquaculture therapeutants, and (4) contaminants and issues of emerging concern. The NCAG has funded a variety of multiyear research projects at Canadian universities and non-for profit research institutions. A summary of the ongoing research projects and their highlights is presented.

Alternative method for collecting epipsammic algae samples from deep water soft sediments (PO)

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Some environmental effects monitoring (EEM) studies have included algae sampling as part of assessing nutrient enrichment effects of pulp mill effluents. River systems, such as the Saskatchewan River, with a low gradient profile, deep water and soft (silt and mud) substrates, can be a challenge to sample for epipsammic algae (attached to grains of sand). These algae can be a major food source for deposit benthic invertebrate feeders such as snails, clams and aquatic worms used to assess pulp mill effects. This study developed an alternative method of collecting algae samples in this type of habitat compared to available sampling methods in the literature. Epipsammic algae were sampled at reference

and exposure (near-field and far-field) area sites with respect to the pulp mill effluent. Initial sampling was attempted with a sediment corer generally used for sampling epipsammic algae, which proved to be unsuccessful due to the consistency of the sediment. In collecting Ekman grabs for sediment samples, it was discovered that, with care, a sample of sediment could be obtained and brought to the surface with the sediment surface layer intact. An area of the sediment surface was delineated by a template which was pressed down into the sediment to a depth of 2-3 mm. The sediment within the template area was then removed by sliding a plastic square along the surface to remove the sediment sitting above the template and placed into sample containers. The algae results obtained using this method of collection were successfully analyzed for trends in algal growth between reference and exposure area sites for chlorophyll a, density, biomass and community composition of major groups.

Epilithic biofilm in outdoor artificial streams as a tool to assess advanced wastewater treatment technologies (PO)

Jose Luis Rodriguez Gil[1] *and Leland Jackson*[1]

[1]*University of Calgary*

An increasing number of studies have shown with certainty that current wastewater treatment processes and technologies fail to remove many chemical compounds we use in our everyday lives. Numerous studies have also shown that some of these compounds affect endocrine function, among others, and can cause dramatic changes in the biological communities of receiving ecosystems. Development of advanced wastewater treatment technologies has closely followed this realization and new technologies are more effective at removing these compounds. Increasing pressure on water resources, with water re-use becoming a real need in many places around the world, makes progress in this field ever more important. Evaluation of the biological effects of these compounds on receiving ecosystems, however, remains limited by difficulties associated with field studies, such as lack of true replication and difficulty selecting reference conditions (even in upstream downstream designs), which limits our understanding of the true biological/ecological effects of these compounds in aquatic communities. These limitations become even greater when attempting to assess and compare differential effects, typically in single systems, from effluents of new wastewater treatment technologies. Now, the 12,320-m artificial streams of the Advancing Canadian Wastewater Assets (ACWA) facility (Calgary, Alberta, Canada) provide a unique platform to answer these questions. Here we present results of biofilm growth in streams that were divided into 4 treatment groups (n=3) representing a negative reference treatment (14 $L \cdot s^{-1}$ of adjacent Bow River water) or one of 3 exposure treatments where Bow River water received a 5% (volume/volume) influx of either final effluent (positive reference) from the Pine Creek Wastewater treatment plant (where ACWA is embedded), or the same volume of this effluent after treatment via in-line advanced oxidation or reverse osmosis. Structural (biomass, chlorophyll a and algal community composition) and functional (community respiration, gross primary production, transformation of organic phosphorus and photosynthetic efficiency) endpoints were assessed in the biofilm communities of the streams to evaluate the effect of the positive reference treatment (Pine Creek final effluent) and whether dosing with effluent from advanced treatment technologies led to any observable changes.

Bioaccumulation of substituted diphenylamine antioxidants and benzotriazole UV stabilizers in an urban creek in Canada (PO)

Zhe Lu[1], Amila De Silva[1], Thomas Peart[1], Cyril Cook[1], Gérald Tétreault[1], Mark Servos[2] and Derek Muir[1]

[1]Environment and Climate Change Canada, [2]University of Waterloo

Substituted diphenylamine antioxidants (SDPAs) and benzotriazole UV stabilizers (BZT-UVs), previously under-reported classes of organic contaminants, were determined in sediment, water, and freshwater biota in an urban creek in Canada. SDPAs and BZT-UVs were frequently detected in all matrices including upstream of the urban area in a rural agricultural/woodlot region, suggesting a ubiquitous presence and bioaccumulation of these emerging contaminants. Spatial comparisons were characterized by higher levels of SDPAs downstream compared with the upstream, implying a possible influence of the urban activities on the antioxidant contamination in the sampling area. In sediment, 4,4'-bis(α,α-dimethylbenzyl)-diphenylamine (diAMS), dioctyl-diphenylamine (C8C8), and dinonyl-diphenylamine (C9C9) were the most dominant congeners of SDPAs, with concentrations up to 191 $ng \cdot g^{-1}$(dry weight). Benthic invertebrates (crayfish (*Orcoescties spp*.)) had larger body burdens of SDPAs and BZT-UVs compared to pelagic fish (hornyhead chub (*Nocomis biguttatus*) and common shiner (*Luxilus cornutus*)) in the creek, and partitioning coefficients demonstrated that sediment was the major reservoir of these contaminants. This is the first report of bioaccumulation and partitioning behaviours of SDPAs and BZT-UVs in freshwater environments.

The cardiorespiratory and metabolic effects of acute naphthalene and pyrene exposure in adult zebrafish (*Danio rerio*) (PO)

Chanel Yeung[1] and Lynn Weber[1]

[1]University of Saskatchewan

Polycyclic aromatic hydrocarbons (PAHs) are aquatic contaminants often originating from anthropogenic sources. Naphthalene (NAP) and pyrene (PYR) are important petrogenic PAHs that are not as well studied as the prototypical PAH, benzo[a]pyrene (BaP). We hypothesized that acute exposure (48 hours) to NAP and PYR will cause sublethal cardiorespiratory and metabolic impairment similar to that observed in previous studies after acute BaP exposure in adult zebrafish (*Danio rerio*), but by different mechanisms. To investigate this hypothesis, adult zebrafish were aqueously exposed to PAHs (NAP, 0, 3.7, 370, and 3700 $\mu g \cdot L^{-1}$; PYR, 0.025, 2.5 and 25 $\mu g \cdot L^{-1}$) using static renewal (24 hours) and compared to dimethysulfoxide controls. No mortalities were observed in any treatment group. At 48 hours, fish (n=16 fish per group) were subjected to high frequency cardiac ultrasound. The ratio of the atrial contractile rate to ventricular rate (AV ratio) and stroke volume (SV) increased in both NAP- and PYR-exposed fish. This higher AV ratio is indicative of an atrioventricular conduction block similar to that observed with BaP in previous studies. However, while the atrial contraction rate for PYR was unchanged compared to control, NAP atrial rate was higher, likely representing an increased adrenergic tone on the heart. In addition, after NAP exposure, a large increase in end diastolic volume (EDV) was noted, but with a lower ejection fraction, indicating some impairment in ventricular contractility despite higher preload mediating great cardiac filling. The higher EDV observed with NAP, but not PYR, may be related to gill irritation and whole-body edema observed with NAP. The acute effects of NAP and PYR on swimming endurance and metabolic rates will be examined and compared to tissue-specific alterations in gene expression to help elucidate differences in adverse outcome pathways. In conclusion, acute aqueous PYR

exposure resembles other AhR agonists, while NAP has additional cardiorespiratory toxic effects that make it a greater potential concern for acute sublethal toxicity in adult fish.

Influence of sunlight on cerium oxide-mediated nanoparticle toxicity in cardinal tetras (PO)

Yueyang Zhang[1] *and Greg Goss*[1]

[1]*University of Alberta*

Most current nanotoxicological studies have been focusing on only a few key model species, such as zebrafish (*Danio rerio*), goldfish (*Carassius auratus*) and trout (e.g., *Oncorhynchus mykiss*). However, the knowledge of the impact of nanoparticles on other aquatic systems is very limited. Cardinal tetras (*Paracheirodon axelrodi*) are a common aquarium freshwater fish found the Rio Negro in the Brazilian Amazon. The Rio Negro has extremely low ion concentrations (~10 µM Ca^2), low pH (~pH<4.5), high natural organic matter (>10 $mg \cdot L^{-1}$) and high intensity sunlight (>2400 $W \cdot cm^{-2}$ UVA daily), giving optimal conditions for CeO_2 toxicity. CeO_2 nanoparticles are a known additive of diesel fuel, and diesel generation is the main source of power in these remote regions. The current study investigates the combined effects of natural organic matter (NOM), CeO_2 nanoparticles and sunlight on the responses of cardinal tetras. CeO_2 nanoparticles were tested at 0.5, 1, 2 and 5 $mg \cdot L^{-1}$ with or without UV-light. Expression of genes related to phase I and II biotransformation enzymes and reactive oxygen species generation and biochemical assays of ion transport and oxidative stress were measured to investigate if sunlight-mediated exacerbation of effects occurs.

Inhalation Toxicology and Air Quality Management

What's that smell? Exploring the factors that influence environmental performance at Alberta's kraft pulp mills (PL)

Nicole Pysh[1]

[1]*Alberta Environment and Parks*

My thesis research explores the factors influencing environmental performance at kraft pulp mills in Alberta, Canada. Total reduced sulfur (TRS) compounds, which have a noxious, rotten-egg odour, were chosen as the indicator of environmental performance because they have a high degree of social intolerance and represent opportunities for process optimization, as they are a by-product of the kraft pulping process. My research had two objectives: first, to determine how the regulatory framework influences TRS emissions management at Alberta's kraft pulp mills; and, second, to gain insights into the influence of corporate environmental management and social licence on environmental performance. Indicators representative of each factor were examined: (1) regulatory conditions, dispersion modelling, point source and ambient TRS monitoring data, (2) voluntary environmental policies and management systems, best management practices, corporate sustainability reporting, and (3) public complaints and ambient TRS exceedances. Extensive literature review, statistical and comparative analysis, and interviews with government and industry subject matter experts were performed. Research results indicate that all factors are influencing performance to varying degrees, but corporate environmental management contributed the most to the variance between facilities. Refining the roles and responsibilities of regulatory bodies, corporate entities, and the public is critical to managing environmental performance and cumulative effects. Recommendations are made to improve the efficacy of all factors in achieving environmental outcomes. Shortcomings in the regulatory framework were identified, but with improvement, it holds the most promise for facilitating collaborative discussions, incentivizing continuous improvement, managing cumulative effects, and enhancing the competiveness of Alberta's kraft pulp industry.

Learnings in developing a regional industrial air emissions management program (PL)

Phoenix Le[1] *and Mary Joy Wesley*[1]

[1]*Government of Alberta*

With concerns over elevated concentrations of fine particulate matter observed throughout the province, particularly in populated areas that are expected to continue to experience population and economic growth, Alberta Environment and Parks is committed to developing a Regional Industrial Air Emissions Management Program aimed at managing and reducing ambient fine particulate matter and its precursors (NO_X, volatile organic compounds, SO_2, and NH_3) from *Environmental Protection and Enhancement Act* (EPEA) approval holders (including Codes of Practice holders). This is the first time that a program of this nature will be used in Alberta. Ambient air quality assessments for 2008-2010 and 2009-2011 showed monitoring stations within the Capital Region exceeded the Canada-wide Standards (CWS) for fine particulate matter. Similarly, in the 2009-2011 and 2010-2012 assessments, an air quality monitoring station in Red Deer also exceeded the CWS for fine particulate matter. In 2012, the Canadian Ambient Air Quality Standards (CAAQS) for fine particulate matter and ground-level ozone replaced the CWS. The first CAAQS assessment results for Alberta for the 2011-2013 reporting period showed Red

Deer Air Zone continued to fail to achieve the CAAQS for fine particulate matter. In addition, all other air zones, except one, were assigned to a management level that required preventative actions be taken to avoid exceeding the CAAQS for fine particulate matter. The objectives of the program are to: (1) better understand and quantify emissions from industrial sources, starting with facilities in Red Deer and North Saskatchewan Air Zones; (2) define baseline control technology currently used in each of the sectors and across industries; (3) ascertain performance optimization of existing equipment, maintenance, and control technology that could lead to further emissions reductions; and (4) ascertain additional viable control technology. The outcomes of this program will: (1) put into action the concept of continuous improvement to inform facilities and the province of the opportunities for emissions reduction through best management practices for on-site operations and equipment upgrades to best available technology; (2) recognize facilities that are environmental stewards who have taken voluntary steps towards continuous improvements; and (3) inform a consistent approach for reducing emissions. Attendees of the presentation will hear why this particular approach was chosen, the learning so far from developing the program, where the program is currently in its development, and anticipated future challenges in developing and implementing the program.

An overview of odour issues in Fort McKay and community initiatives to understand and address these issues (PL)

David Spink[1], John Dennis[2] and Ryan Abel[3]

[1]Pravid Environmental Inc., [2]SolAeroMed Inc., [3]Fort McKay Sustainability Department

The First Nation and Métis community of Fort McKay is situated in the middle of the mineable portion of the Athabasca Oil Sands Region. One of the impacts associated with this oil sands development is odours, and oil sands-related odours have been an issue and concern for Fort McKay going back to the 1980s. One of the concerns linked to odours in particular, and to overall air quality in general, is their possible impact on health. The federal government notes that, “Air pollution has significant negative effects on human health” and the World Health Organization indicates that, “Clean air is considered to be a basic requirement for human health and well-being.” For most people, the quality of air and its perceived safeness is judged by sensory perception (i.e., visibility, taste and smell). Odours are on the most frequent air quality complaint issues and can have both direct and indirect health impacts. The Royal Society of Canada, in a 2010 report, noted that for Fort McKay, “Odour is certainly recognized as a problem for this community. Although odour has often been considered a nuisance rather than a health effect, chronic odour problems become a burden on community well-being which ultimately leads to stress with the possibility of associated health effects. Resolution of the odour problems being caused by oil sands developments is clearly necessary.” The approval process for oil sands projects involves an odour impact assessment. These assessments generally show “none” to very limited potential for odour impacts, which is inconsistent with the “real world” experience of the community. Approvals for oil sands projects have very basic odour-related emission and ambient monitoring requirements, if any. Odour levels in Fort McKay, as inferred from continuous total reduced sulfur monitoring data, have fluctuated over the last 16 years. Elevated odour levels in the 2006-2010 period (some severe odour episodes during this period) and perceived deficiencies in the monitoring and assessment of odours resulted in Fort McKay undertaking a number of odour-related programs and activities. These included: air quality canister sampling (since 2010) during certain odour events to identify the compounds responsible for the odours being experienced, their source(s) and whether or not air quality during odour events posed a health risk; installation of two eNose monitors in the community to continuously measure odour levels; development of odour impacts assessment guidelines

to try to enhance and improve odour impact assessments; development of an odour event attribution protocol to try to link an odour event to a specific source type and/or source(s); and, studying available emission profiles for various emission sources in relation to canister sampling chemical profiles to identify possible priority sources for management in terms of odorant emission releases.

The presentation will provide an overview of the results and status of some of these initiatives, with a focus on air quality measurements during events at Fort McKay relative to established odour thresholds and acute toxicity screening levels. Gaps in the current monitoring, assessment and regulatory processes related to odours will be identified and possible options for addressing these gaps will be suggested.

Controlling odours in the Peace River area (PL)

Michael Zelensky[1]

[1]*Albert Energy Regulator*

Oil and gas development has been occurring in the Peace River area since the late 1950s; however, it hasn't been until the last decade that technological advances have made development of the bitumen in the area economically feasible. Since then, industry activity has increased, as has the volume of odour complaints from area residents. Despite significant multi-stakeholder efforts to resolve odour and emission problems, the complaints continued. A proceeding was initiated to gather information from area stakeholders and subject matter experts, which included an organizational meeting and an eight-day hearing in Peace River, Alberta, starting on January 21, 2014. The Panel carefully considered the information it received and provided the following recommendations for operations, air quality monitoring and health concerns in their March 31, 2014 report:

A) Operations: Practical operational measures should be implemented to capture and conserve gas. Venting should be eliminated and produced gas should be captured using vapour recovery units (VRUs) within four months in the Reno and Three Creeks areas. The Panel recommended that studies be conducted with respect to the installation of VRUs in the Walrus and Seal Lake areas, as well as options and timelines for conserving all produced gas in the Peace River area. The Panel also recommended the implementation of measures to minimize odours from trucks, as well as practices to identify fugitive emissions and address them expeditiously.

B) Monitoring: The Panel recognized that, despite significant efforts to monitor air quality in the Peace River area, there has been little correlation of the results of air monitoring with the odour events reported by residents in the area. There has also been a lack of communication of such results to area residents in a clear and understandable manner. The Panel's main recommendation in this matter was to establish a comprehensive and credible regional air quality monitoring program for the Peace River area that will verify, through reliable and accessible data, that the recommended operational changes have improved air quality.

C) Health: The Panel's main finding was that odours from heavy oil operations in the Peace River area have the potential to cause some of the symptoms experienced by residents; therefore, these odours should be eliminated. The Panel recommended that further study be conducted to examine linkages between odours and emissions and health effects.

This paper will provide a status update on these three areas of concern. Progress on installing operational controls on emissions and their effectiveness will be summarized. Continuous

monitoring results from the Peace River Area Monitoring Program (PRAMP) will be presented to demonstrate the improvement in air quality. Results from triggered canister sampling for volatile organic compounds and reduced sulfur compounds will be compared to threshold limits for health effects.

Comparative toxicity of airborne particulate matter collected in the vicinity of industrial sites (PL)

Errol Thomson[1]

[1]*Health Canada*

Airborne particulate matter levels are linked to adverse health outcomes, including cardiovascular and respiratory morbidity and mortality. Industrial emissions are important contributors to national particulate air pollution levels, and emissions from different industrial sources can vary in their chemical composition, which in turn may impact the toxicity of the material. To assess the relative potency of particles from distinct sources, we have conducted studies using cell culture models exposed to size-fractionated particles collected in the vicinity of specific industrial sites (steel, copper, aluminum, petrochemical), and compared effects across a range of cytotoxicity and inflammatory assays. Particles displayed striking source-dependent differences in chemical composition and in their toxicity to cultured cells. Particle toxicity was associated with particle size and with the metal, polycyclic aromatic hydrocarbon, and endotoxin content of the particles. These results are consistent with the hypothesis that health effects of particles depend not only on mass concentration but also on source-dependent changes in composition. Investigations conducted *in vivo* have identified activation of several biological pathways, including the stress response system, which could contribute to adverse health impacts of inhaled pollutants. A better understanding of how sources contribute to particulate matter toxicity through differential perturbation of biological processes will help guide regulatory efforts aimed at reducing the health burden attributed to exposure to particulate air pollution.

Advances in Water Quality Guidelines and Benchmarks

International perspective on water quality benchmarks (PL)

Uwe Schneider[1]

[1]*Water Quality in the 21 Century*

The second International Conference on Deriving Environmental Quality Standards (EQS) for the Protection of Aquatic Ecosystems (EQSPAE 2016) was held on June 18-20, 2016 in Hong Kong. It was attended by over 120 scientists from four continents, presenting and discussing current issues of EQS development. In the first part of my talk, I will present some of these issues, results, and activities from EQSPAE relevant to water quality guideline development in Canada. In the second part, I will compare some select water quality standard derivation methods from other countries relative to Canada's, and point out some potential areas of cooperation, improvement, and problems with the Canadian Water Quality Guidelines for the Protection of Aquatic Life.

Current status and future directions for Canadian Environmental Quality Guidelines (PL)

Joanne Little[1]*, Doug Spry*[2]*, Tamzin El-Fityani*[2]*, Tim Fletcher*[3] *and Monica Nowierski*[3]

[1]*Alberta Environment and Parks,* [2]*Environment and Climate Change Canada,* [3]*Ontario Ministry of the Environment and Climate Change*

The Canadian Council of Ministers of the Environment (CCME) has been the primary interjurisdictional forum for development of Canadian environmental quality guideline (CEQG) development. CEQGs for the protection of aquatic life are currently developed under the guidance of the Guidelines Project Team. Guidelines were recently published for silver and are currently under development for zinc, manganese and carbamazepine. The presentation will highlight some of the challenges in the development of these guidelines, including accounting for multiple toxicity modifying factors, nuances in the application of the CCME (2007) protocol, assessing the suitability of toxicity data, and ensuring an adequate level of protection. In addition, future priorities and challenges for guideline development, including use of novel endpoints, potential adoption of guidelines from alternate sources and incorporation of bioavailability, will be outlined.

The importance of updating water quality guidelines (PL)

Charlene Burnett-Seidel[1]

[1]*Cameco Corporation*

Water quality guidelines can be useful tools for assessing the potential risk to aquatic organisms. The review and re-derivation of guidelines have not kept up with the state of science for many substances. Some national guidelines adopted by other jurisdictions in Canada are nearly 30 years old. In some cases, data that formed the basis of a guideline are not reproducible using current standard procedures. The methods used to derive water quality guidelines have evolved. The use of a more robust approach such as the species sensitivity distribution is now preferred over the use of the safety factor approach. As a result of these issues, many guidelines are out of sync with the current risk context and decisions are

being made with out-of-date guidelines. In many cases, studies to address gaps in toxicity data are typically routine and cost-effective. As we move towards a new era for water quality guidelines, there is a need for a collaborative approach between industry, government, academia, and the public to ensure that guidelines are up-to-date and that potential risk to aquatic organisms is characterized and communicated appropriately. Using this collaborative approach in combination with new science and derivation methods, updating water quality guidelines can be accomplished successfully. The benefits of updating guidelines apply to all stakeholders (communities, companies, governments).

Federal Environmental Quality Guidelines: Recent progress (PL)

Doug Spry[1], Philippa Cureton[1] and Sushil Dixit[1]

[1]Environment and Climate Change Canada

Under the *Canadian Environmental Protection Act, 1999*, the Government of Canada has the authority to develop Federal Environmental Quality Guidelines (FEQGs), which provide benchmarks for the quality of the ambient environment. Where the FEQGs are met, there is low likelihood of adverse effects on the protected use (e.g., aquatic life or the wildlife that may consume them). They are based on the toxicological effects or hazards of specific substances or groups of substances and do not take into account analytical capability or socio-economic factors. FEQGs follow the four existing Canadian Council of Ministers of the Environment (CCME) protocols for protection of aquatic life, sediment, soils and tissue residue to the greatest extent possible. FEQGs can play several roles. Many of these are closely related to the Chemicals Management Plan, including: supporting federal water quality monitoring programs, risk assessments, and risk management activities. FEQGs can aid in preventing pollution by providing targets for acceptable environmental quality, help evaluate the significance of concentrations of chemical substances currently found in the environment (monitoring of water, sediment, and biological tissue), and serve as performance measures of the success of risk management activities. The most recent group of FEQGs was notified in *Canada Gazette* on May 28, 2016. Included were chlorinated alkanes, vanadium, tetrabromobisphenol A (TBBPA) and hexabromocyclododecane (HBCD), and, along with earlier FEQGs, they are available online. The FEQG process will be discussed using Bisphenol A (BPA) as an example that is currently under development.

British Columbia *Contaminated Sites Regulation* update 2016/2017 (PL)

Aimee Brisebois[1] and Jennifer Puhallo[1]

[1]British Columbia Ministry of Environment

Many of the numerical environmental quality standards listed in British Columbia's *Contaminated Sites Regulation* (the Regulation) have not been updated since the Regulation came into effect in 1997. Although eight of the nine previous amendments to the Regulation have included changes to some of the numerical standards, these changes have generally reflected the addition of new standards to address new environmental media (e.g., addition of sediment quality criteria in 2004 and vapour quality standards in 2009) or the addition of standards for new substances not previously regulated (e.g., new human health soil quality standards and drinking water standards for the substances of Schedule 10 of the Regulation), and none of the previous revisions to the standards have constituted a comprehensive review. Over the past two years, the British Columbia Ministry of Environment's Land Remediation Section has completed the first comprehensive review, revision and re-verification of all the standards of

the Regulation conducted since the Regulation came into effect. This work has addressed new science, including new human and ecological toxicology modifiers, toxicity reference values, exposure scenarios, hydrogeological modelling and new scientific methodology related to the toxicological derivation of updated new environmental quality standards for water, vapour and soil for use in the management of contaminated sites in the province under a proposed tenth amendment to the Regulation. In our presentation, we will provide an overview of some of the successes and challenges faced in revising and updating the environmental quality standards for the proposed Stage 10 amendment to the *Contaminated Sites Regulation* and offer some important "lessons learned". We will also highlight both some of the substances and the methods and steps used to derive proposed new environmental quality standards for several "emerging contaminants of concern" that have been proposed for inclusion in the Stage 10 amendment.

New approaches to deriving species sensitivity distributions to inform water quality guidelines (PL)

Angeline Tillmanns[1] and Carl Schwarz[2]

[1]British Columbia Ministry of Environment, [2]Simon Fraser University

Species sensitivity distributions (SSDs) are probabilistic models that estimate species sensitivities to a contaminant of concern. The modeled distribution allows the extrapolation of a hazard concentration that can be considered protective of 95% of species (HC5). Nationally, SSD is the primary method used to develop water quality guidelines. The method has also been adopted by multiple international jurisdictions including the European Union, Australia and New Zealand. As with all models, there are a number of assumptions and uncertainties in the SSD approach. Different jurisdictions have taken slightly different approaches to SSD, highlighting the latitude available in the multiple steps that go into developing a final HC5. These steps include selecting data, identifying an appropriate model and calculating uncertainties. A major assumption of the first step is that laboratory-generated toxicity data are representative of field conditions. This is an assumption of all methods to generate guidelines, but the SSD offers unique opportunities to include the intra-specific variation of a species by including data for unique genotypes. A second major assumption is that species sensitivity data follow a single continuous distribution. In the Canadian Council of Ministers of the Environment (CCME) protocol, univariate toxicity data are fit to a number of potential distributions by inferring a plotting position (location on the y-axis) and then using regression type methods to assess the goodness of fit and select a continuous probability distribution. An alternative approach is to use maximum likelihood estimation (MLE) techniques to estimate parameters directly and then assess goodness of fit for a number of distributions. MLE utilizes the data directly so there is no need to decide on a plotting position for the y-axis. Also, given the small data sets, a number of distributions may fit equally well. Using MLE and information theoretic methods, a weighted average can be generated to combine multiple models. Model and extrapolation uncertainties can also be calculated using MLE. The widely utilized free statistical package, R, allows the use of modern statistical methods to determine the best model(s) and calculate uncertainty estimates. Fitting distribution models to species sensitivity data is an evolving science. In this presentation, a number of new ideas are explored with the intention of reducing the potential error associated with underlying assumptions and associated uncertainties.

Perspectives on the appropriate use of laboratory toxicity test information in deriving water quality guidelines and incorporating toxicity-modifying factors (PL)

James Elphick[1], Adrian deBruyn[2] and Curtis Eickhoff[1]

[1]Nautilus Environmental, [2]Golder Associates Ltd.

Derivation of water quality guidelines requires the use of results from laboratory toxicity tests. In Canada, long-term exposure guidelines are ideally calculated using a species sensitivity distribution (SSD) of "no effect" concentrations from chronic toxicity tests conducted in the laboratory. The preferred endpoint from these tests is the IC_{10} or EC_{10} value; however, there is often considerable uncertainty in the derivation of these point estimates, which are in the tail of the concentration-response curve. Furthermore, these endpoints are not readily available for many data sets. This presentation discusses aspects of study design to optimize the value of the information obtained from toxicity tests; addresses challenges in calculation of IC_{10} values and discusses alternative approaches to calculating no effect levels; and provides recommendations on methods for inclusion of exposure and toxicity modifying factors in water quality guideline derivations.

Development of a proposed Canadian Water Quality Guideline for molybdenum (PL)

James Elphick[1], Brett Lucas[1], Claudio Quinteros[1] and Charlene Burnett-Seidel[2]

[1]Nautilus Environmental, [2]Cameco Corporation

A revised, long-term Canadian Water Quality Guideline for the Protection of Aquatic Life was calculated for molybdenum using the species sensitivity distribution (SSD) approach and based on an updated database of freshwater toxicity data. This data set included information available in the scientific literature that met good scientific standards, as well as new data produced for the oligochaete *Tubifex tubifex*, to validate a low acute endpoint reported in the literature, and for brown trout (*Salmo trutta*) to produce novel data for a second salmonid species. The *T. tubifex* test was a 96-hour survival test, whereas the brown trout test was an 85-day development test that investigated the effects of molybdenum over the embryo, alevin and fry life-stages. Results from the *T. tubifex* test suggested that previous results may have overestimated the toxicity of molybdenum to this species, as a 96-hour LC_{50} of 2,782 $mg \cdot L^{-1}$ was determined, two orders of magnitude higher than the original test. Brown trout were also determined to be relatively insensitive to molybdenum, and produced an EC_{10} value for growth of 202.5 $mg \cdot L^{-1}$. A new long-term guideline was determined using the 5^{th} percentile of an SSD that included data for eleven species, including three species of fish, five invertebrates, two plants/algae, and one amphibian. Based on these results, a new long-term guideline of 26 $mg \cdot L^{-1}$ is proposed.

Water hardness and chloride toxicity towards aquatic life (PL)

Anthony Knafla[1] and Ian McIvor[1]

[1]Equilibrium Environmental Inc.

In 2011, the Canadian Water Quality Guidelines for the Protection of Aquatic Life for chloride were revised to incorporate new toxicological research including assays conducted on sensitive bivalve life-stages. While water hardness was identified by the Canadian Council of Ministers of the Environment (CCME) as an important factor modifying chloride toxicity, it was not incorporated, partly because of a

lack of suitable data. A supplementary literature review was conducted covering years up to 2016, and additional studies were identified for incorporation into chloride guideline calculations and for assessing the effects of hardness. These additional literature data were further supplemented with new toxicity testing assays that were commissioned, using several sensitive aquatic species. Revised short-term and long-term toxicity benchmarks were subsequently derived using a species sensitivity distribution (SSD) approach. The results suggest that current Canadian Water Quality Guidelines for the Protection of Aquatic Life for chloride may be insufficiently protective in soft water environments, while being too conservative in moderately hard to very hard conditions. Furthermore, there are aspects of toxicity related to different combinations of cations, which should be considered when evaluating adverse effects associated with chloride exposure to aquatic life species, in the presence of varying water hardness.

Initial steps towards updating British Columbia's water quality guideline for copper (PL)

Ali Azizishirazi[1], Angeline Tillmanns[2] and Greg Pyle[1]

[1]University of Lethbridge, [2]British Columbia Ministry of Environment

Copper is one of the most well-studied contaminants globally due to its geographic ubiquity, abundance, and toxic effects that occur at relatively low concentrations. Copper guidelines in Canada are almost 30 years old. Both the Canadian Council of Ministers of the Environment (CCME) and British Columbia's (BC's) water quality guidelines (WQG) were published in 1987 and are hardness-based. Since 1987, many papers have been published on different aspects of copper toxicity and our knowledge about adverse effects of copper has changed significantly. As an example, many studies have demonstrated that the ameliorative role of water hardness is variable depending on a variety of other water quality factors such as alkalinity. In addition, many other water quality factors (such as dissolved organic carbon, alkalinity and pH) have been demonstrated to affect copper toxicity. Furthermore, knowledge of sublethal effects of copper at environmentally relevant concentrations has been enormously expanded. Therefore, there is a simultaneous need and opportunity to review the toxicological literature in support of updating copper guidelines. In the process of conducting the review, more than 6000 studies investigating adverse effects of copper to aquatic organisms have been screened against BC Ministry of Environment inclusion criteria, which resulted in more than 1200 papers flagged for detailed review. These papers were categorized on the basis of taxonomic group. Representative studies for some taxa were surprisingly lacking (i.e., amphibians). About 320 papers were categorized as "primary" or "secondary" based on screening criteria. Despite the sheer number of investigations published since 1987, there are still knowledge gaps in our understanding of copper toxicity to aquatic organisms, and recommendations will be provided to researchers to fill them.

Industrial Development and the Potential Environmental and Health Implications for Aboriginal Communities

Direct and indirect impacts from industrial emissions on the aboriginal community of Fort McKay, Alberta (PL)

John Dennis[1], David Spink[2], Ryan Abel[3] and Bori Arrobo[3]

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The World Health Organization (WHO) defines "health" as a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity. Canadian provincial and federal systems assess and regulate industrial emissions with a view to minimizing direct impacts ranging from potential for human exposure to acute and chronic health impacts. This process is generally managed well and incorporates safety factors and precautionary principles. There are many human health impacts that remain invisible to these environmental regulatory systems but continue to harm our communities, with perhaps greatest impact on Aboriginal communities. For example, a major issue affecting Aboriginal health in Fort McKay includes poor diet, particularly with increased reliance on western, store-bought foods. Affordable food choices in western groceries lead to obesity, diabetes and other health issues. Traditional Aboriginal foods offer better nutrition, and their collection promotes physical, mental, and community well-being. The perception of industrial emissions contaminating traditional foods is pervasive in many Aboriginal communities, including Fort McKay First Nation located in midst of major oil sands developments in northeastern Alberta. The perception of high levels of contamination is reinforced through industrial odours, visible stack emissions, and regular reports of plant malfunctions leading to air and water emissions. These perceptions manipulate the community's members away from traditional foods and inadvertently promote consumption of western store bought foods. Decreased traditional land use further conspires to erode individual and community health, which compounds indirect health impacts and links to mental health and addictions. If our regulatory systems were better able to identify and accept indirect links between emissions and health impacts, it would allow mitigations that better manage perceptions (e.g., through education and capacity building, effective communication tools), which could reduce fear and promote greater gathering and consumption of traditional foods. In turn, this would result in increased physical and mental health for individuals and communities. The current provincial regulatory system focuses almost exclusively on direct health impacts and is not serving our Aboriginal community well. More mature health impact systems are evident in federal Canadian and U.S. systems, as well as internationally through the WHO and the United Nations. The provincial system should be encouraged to evolve to be better able to identify, assess, and mitigate indirect impacts from industrial development. Only in this way will the tools be provided for our Aboriginal population to help address the serious health and well-being issues faced in Fort McKay and most other First Nations. Revising our regulatory systems to include indirect impacts inherent in ecosystem health would benefit all our Aboriginal peoples, as well as the whole of Canada.

Unifying Aboriginal knowledge and science to support community-based monitoring in a large river and delta (PL)

Lorne E. Doig[1], Tim Jardine[1], Chrystal Mantyka-Pringle[1], Lori Bradford[1], Lalita Bharadwaj[1], Jennifer Fresque-Baxter[2], Erin Kelly[2], Gila Somers[2], Karl-Erich Lindenschmidt[1], Paul Jones[1] and Slave River and Delta Partnership

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Cumulative effects, driven in part by anthropogenic stressors, lead to disproportionate effects on Aboriginal communities that are reliant on land, wildlife and water. Understanding and counteracting these effects requires knowledge from multiple sources. Yet the combined use of traditional knowledge and science has both technical and philosophical hurdles to overcome, and suffers from inherent power dynamics that disfavor the very communities it intends to benefit. Here we show how the development of a monitoring program to assess environmental change in the Slave River and Delta (Northwest Territories, Canada), which was initiated and guided by the community, can minimize many of the challenges commonly observed. First, community ownership and the articulation of key guiding questions dictated all indicators that were to be measured in the two-year program. Second, we used a Bayesian Belief Network that balanced science (biophysical measurements) and traditional knowledge (stories of change told through interviews) indicators to create a shared understanding of the system. Third, we used a variety of dissemination tools, including a whiteboard animation of elders' stories, to communicate the results in more meaningful ways. Through this approach we hope to have created a more sustainable monitoring program that will now be pursued by the community. While many challenges remain, we believe that adapting this framework elsewhere could yield better information on environmental change, and a better representation of the knowledge and values of affected communities.

Using inputs from an Indigenous community in evaluating remedial options at a large mine site (PL)

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Unique approaches to conducting a human health and ecological risk assessment based on more realistic exposure scenarios will be discussed, using a former lead/zinc mining site in the Yukon as an example. This site has numerous adits, tailings, and waste rock piles across a 15,000-hectare area and involves several different watersheds. A remedial action plan has been developed for the site and the risk assessment focused on residual risks after completion of the remedial activities. Currently, the majority of the water on the site is treated and it is proposed to install a new water treatment plant to improve performance and reliability. The post-remediation conditions for the terrestrial environment involve the removal or covering of tailings and waste rock piles and replacement with clean till. As part of the problem formulation development for the risk assessment, a community consultation session was held with the Indigenous community that is located near to the site. The session was used to discuss elements of the risk assessment, and to collect information for use in the risk assessment on locations used for drinking water, fishing, hunting, and gathering, as well as types of food consumed. A watershed approach was used in the assessment, taking into account the unique contamination profiles, expected reclamation activities, and anticipated future land uses at different exposure locations within each watershed. For the evaluation of wildlife and humans, a spatial averaging approach was used to develop

the soil and terrestrial vegetation exposure point concentrations, accounting for the remedial activities in the different watersheds. Within the watersheds, smaller exposure areas were also evaluated to help determine whether additional remedial activities would be needed to further reduce risks. The results of the assessment were discussed with the community.

The influence of hydropeaking on growth and mercury concentrations of low-pitched shoreline dwelling spottail shiner (*Notropis hudsonius*) (PL)

Derek Green[1], David Janz[1], Tim Jardine[1] and Lynn Weber[2]

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Hydroelectric reservoir construction induces chemical and physical changes on impounded waters that affect local ecosystems. Among this suite of changes are those that favor the production of neurotoxic methylmercury, which can subsequently bioaccumulate in impounded and downstream fish. In the 1970s, concerns arose about mercury contamination in fish caught by the Aboriginal fishery of Cumberland House, located downstream of the E. B. Campbell hydroelectric dam (est. 1963) in Saskatchewan, Canada. Elevated mercury concentrations ([Hg]) were observed in filets of northern pike (*Esox lucius*), walleye (*Sander vitreus*), goldeye (*Hiodon alosoides*), and sauger (*Sander canadensis*) and commonly exceeded consumption guidelines (0.5 $mg \cdot kg^{-1}$) in both the fishery and reservoir of the dam. While current [Hg] are now below consumption guidelines in these populations, the decline was significantly slower in downstream walleye and goldeye ($p<0.001$). These trends were found despite low [Hg] in contemporary water samples collected immediately below the dam, implicating elements of the downstream environment as causal drivers of Hg retention in fish. Observed fish kills and strandings on low-pitched shorelines below the dam suggest that the irregular and highly variable water levels observed downstream of this peaking hydro-electric facility may act as a chronic physical stressor, causing increased retention of Hg in downstream fish by diminishing growth dilution in some species. Analyses of triglyceride concentrations and condition factor (K) of young of the year spottail shiner (*Notropis hudsonius*) collected from affected habitats reveal reduced energy accumulation and condition in young of the year fish ($p<0.05$) and relatively greater [Hg] ($p<0.05$) compared to upstream counterparts, suggesting that peaking hydro-electric facilities may induce an energetic bottleneck that exacerbates [Hg] in downstream fish. Analyses of glycogen depletion and cortisol induction in response to an acute physical stressor are currently underway to determine whether fish from affected habitats exhibit signs of chronic stress.

New Methods and Novel Approaches for Assessing and Monitoring Environmental Contaminants

Ahead by a century: Laboratory research to support the development of a standardized test method using a native amphibian (PL)

Bonnie Lo[1], Paula Jackman[2] and Leana Van der Vliet[2]

[1]Nautilus Environmental, [2]Environment and Climate Change Canada

Xenopus laevis, a tropical species non-native to Canada, has dominated standardized laboratory-based amphibian testing for decades. While there are many advantages to working with *Xenopus* species, there is considerable doubt that this species is a suitable surrogate for Canadian amphibian species. Working towards a standardized test method with a native amphibian species (*Lithobates pipiens*, northern leopard frog) has provided many challenges over the last 10+years, and here we present our most recent research. Reviews of the literature and our own laboratory experience have shown that leopard frog tadpoles can survive and grow on many different diets. However, the challenge for the test method was not rudimentary survival and growth, but optimization and efficiency. Results from recent feeding trials will be reviewed, including decisions made at the end of the test to simplify the most effective feeding regime. The typical reference toxicity test design (96-hour lethality) is expected to be significantly altered for the leopard frog test, both to (i) more closely match the endpoints of the primary test, which are growth and development, and (ii) to reduce number of organisms used in this vertebrate test. Multi-concentration longer-term tests with two candidate reference toxicants (sodium chloride and thyroxine) were performed to inform our decisions in the re-design of the reference toxicant test. We will describe the rationale for the choice of our candidate reference toxicants, why and how the goals of this testing are distinct from a short-term lethality test, and examine the results in light of the proposed new paradigm for positive controls. Environment and Climate Change Canada's amphibian test method will continue along the usual path for validation of a toxicity test method, including an inter-laboratory study, development of quality control guidelines, and detailed test method text. The result will be the first standardized test method using a native amphibian species—if not showing we are ahead by a century, then by at least a decade.

Development and validation of a standardized amphibian test method using the northern leopard frog, *Lithobates pipiens* (PL)

Jennifer Miller[1], Leana Van der Vliet[2], Lisa Taylor[2] and Rick Scroggins[2]

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Although there is growing evidence of their sensitivity to contaminants, and growing demand for their use in regulatory frameworks, amphibian toxicity data are currently under-represented in risk assessments. Few standardized methods are available, which contributes to this under-representation, and none of the available methods pair whole-organism chronic endpoints with species that are relevant to Canadian environments. For over 10 years, Environment and Climate Change Canada has sponsored research for the development and standardization of an amphibian toxicity test method using *Lithobates pipiens* (northern leopard frog, formerly *Rana pipiens*), with a focus on sublethal, chronic effects (growth

and development) which manifest as a result of whole-organism, aqueous exposure. We will describe the scope and key aspects of the new test method, including: acquiring, holding and rearing of test organisms, the use of two distinct exposure periods, considerations to address animal care and the "3 Rs" (replacement, reduction, refinement), a new proposed approach for positive control testing, overview of recent method development research, planned inter-laboratory study, and progress towards test method completion. Once released, this standardized amphibian toxicity test method could be used in risk assessments, contaminated site assessments, and in pesticide regulation and registration. Additionally, results from chemical-specific tests could be incorporated into Canadian water quality guidelines.

How to assess risk from contaminated sites to amphibians (PL)

Francois Cloutier[1]

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The Federal Contaminated Sites Action Plan (FCSAP) supports site managers in reducing human health risks, ecological risks and financial liabilities associated with federal contaminated sites. FCSAP provides guidance for ecological risk assessment that promotes applying a comprehensive weight of evidence approach to assess risk from contaminants to all receptors, including amphibians. Risk to receptors at federal contaminated sites is investigated through different lines of evidence: (1) conducting site-specific toxicity tests; (2) comparing exposure at the contaminated site to literature-based toxicity data; (3) conducting site-specific biological field studies; and (4) comparing site-specific exposure to biological field studies reported in the scientific literature (FCSAP 2012). Until recently, amphibians have often been excluded from risk assessments because site-specific methods or literature data are either not available or not easily accessible. In order to facilitate risk assessments for amphibians, Environment and Climate Change Canada is developing practical guidance for amphibian risk assessments for the four different lines of evidence. For site-specific toxicity and biological testing, available methods are being evaluated and provided to the risk assessor. For comparing site-specific contaminant concentrations to concentrations in laboratory-based toxicity studies, amphibian toxicity concentration-response data are being compiled for selected contaminants. Amphibian toxicity data for lead, cadmium, zinc and mercury covering multiple endpoints are illustrated as multi-study concentration-response relationships. This allows the risk assessor to go beyond point-estimate based hazard quotients and evaluate risk in context of effect magnitude and uncertainty across a range of concentrations. The concentration-response data compilations also confirm whether existing water quality guidelines, which are typically developed without specific amphibian considerations, are providing adequate protection for this sensitive group of receptors.

Mollusca representation in species sensitivity distributions: The use of the ramshorn snail (*Planorbella pilsbryi*) in toxicity testing (PL)

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The phylum Mollusca often goes unrepresented in species sensitivity distributions used to characterize the effect of a chemical in ecological risk assessments. Mollusks have the greatest number

of documented extinctions of any major taxonomic group: 42% of recorded extinctions since the year 1500. The International Union for Conservation of Nature's list of threatened species contains 708 species of freshwater mollusk. Therefore, should Mollusca be represented in species sensitivity distributions? The current study investigated how a freshwater pulmonate snail (*Planorbella pilsbryi*) that is native to North America and found across Canada could be used in assessing the potential hazard of a chemical to aquatic ecosystems. This species is relatively simple to culture in the laboratory and allows for an assessment of effects on mortality and growth at different life-stages and on reproduction. This study specifically examined the effect of the surfactant MON 0818, present in formulations of the herbicide Roundup®, on embryonic, juvenile, and adult snails. Environmentally relevant concentrations of MON 0818 would not cause significant mortality in the different life-stages of the snail but did cause inhibition of oviposition in adult snails.

Toxicity of polychlorinated biphenyls and N-phenyl-1-naphthylamine in juvenile turtle, *Chelydra serpentina* (PL)

Tash-Lynn Colson[1], Shane de Solla[2] and Valerie Langlois[3]

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Polychlorinated biphenyls (PCBs) as well as substituted phenylamines (SPAs) antioxidant are two chemical groups that have been used in multiple Canadian industrial processes. Despite the ban of PCB production in North America in 1977, they are still ubiquitously found in the environment and have body burdens in wildlife. Previous studies with mammals, birds, amphibians and fish have shown PCBs to be neurotoxic, genotoxic, teratogenic, and have been classified as endocrine disruptors. In contrast, SPAs, specifically N-phenyl-1-naphthylamine (PANA), have received very little attention despite their current use in Canada and their expected aquatic and environmental releases. There is a research gap regarding the effects of PCBs in reptiles and PANA in wildlife; therefore, snapping turtle (*Chelydra serpentina*) was studied due to its importance as an environmental indicator. The first experiment was conducted using food pellets spiked at an environmentally relevant concentration of the PCB mixture Aroclor 1254 (A1254) to model bioaccumulation and depuration of PCBs in the turtle's liver. Turtles were fed food contaminated with 500 $ng \cdot g^{-1}$ A1254 for 31 days, followed by clean food for 50 days. No significant differences were observed between the control and treated animals. This suggests that juvenile turtles exposed to food items contaminated with 500 $ng \cdot g^{-1}$ PCBs are capable of metabolizing PCBs at a fast enough rate as to avoid bioaccumulation. Two additional dose-response experiments were performed using A1254 and PANA spiked food to determine hepatic toxicity and bioaccumulation in juvenile *C. serpentina* (0-12,500 $ng \cdot g^{-1}$ and 0-10,000 $ng \cdot g^{-1}$, respectively). An increase in cyp1a was observed when exposed to the highest dose of A1254 correlating to the significant increase in hepatic PCB congeners that are known to be metabolized by CYP1A. PCBs are known endocrine disruptors, but herein, although non-significant increasing trends were observed for both thyroid receptors alpha and beta, no changes were found for both the estrogen and androgen receptors. This lack of response suggests that *C. serpentina* is less sensitive to PCB endocrine disruption than other species. Similarly to PCBs, a significant increase in cyp1a mRNA expression was also observed in the turtle liver when exposed to the highest dose of PANA, which is also suggesting its potential role in contaminant metabolism. Additionally, a suite of cellular stress genes was studied for both PCB- and PANA-exposed animals, but none of the genes were altered by any treatments further supporting the resilience of turtles to oxidative stress. Overall, this study has demonstrated the toxicity of a persistent and an emergent contaminant, which will help monitor and predict health risks associated with environmental contamination for *C. serpentina* populations.

New endpoint of tail length in fathead minnow embryo-larval tests (PL)

Joanne Parrott[1], Cheryl Sullivan[1], Kallie Shires[1], Christine Lavalle[1] and Quintin Rochfort[1]

[1]Environment and Climate Change Canada

Seven-day fathead minnow (*Pimephales promelas*) larval survival and growth tests are useful for the detection of acute toxic effects of chemicals and effluents. We have extended the test adding 5 days at the beginning (egg stage) and 9 days at the end (late larval early juvenile stage). The 21-day embryo-larval exposure starts with fertilized fathead minnow eggs, and continues through hatching, sub-sampling of the larval fish at 9 days post-hatch, and ending at 16 days post-hatch. We have used it to study the effects of polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs, and oil sands-related compounds, as well as to pure chemicals (azo dyes and substituted phenyl amines) tested as part of Canada's Chemicals Management Plan. It can be used with waterborne chemicals/samples and sediment exposures. One recent addition to the test was use of the endpoint tail length in larval fish at 9 and 16 days post-hatch. A series of PAH-containing sediments from a Great Lakes Area of Concern (Randle Reef in Hamilton Harbour) was assessed in the 21-day test. While only one of 12 sediments was overtly toxic, 4 sediments reduced growth of fish. Of the growth reductions, most of the decreases in length of larval fish at 9 and 16 days post-hatch were caused by smaller tail lengths. In cases where sediment exposure decreased larval fish length by 9-20%, tail length was decreased by 36-54% compared to control sediment-exposed fish. Similar reductions in tail length were seen in embryo-larval exposures to some river sediments (containing alkylated PAHs and PAHs) from the oil sands area of Alberta. The measurement of tail length provides an additional, sometimes sensitive, endpoint to the 21-day fathead minnow embryo larval test.

Assessing the lethal and sublethal toxicity of neonicotinoid insecticides to the mayfly, *Hexagenia* spp. (PL)

Adrienne Bartlett[1], Amanda Hedges[1], Lisa Brown[1], John Struger[1] and Shane de Solla[1]

[1]Environment and Climate Change Canada

Neonicotinoids are the most widely used insecticides in the world. They are preferentially toxic to insects while displaying a low toxicity toward vertebrates; this selective toxicity has led to their rapid and ubiquitous use. However, neonicotinoids may negatively affect aquatic ecosystems because they are environmentally persistent and highly water-soluble, and thus are prone to leaching from the soil into surface waters via run-off events. Although non-target aquatic organisms may be adversely affected by these compounds, few data are available regarding the effects of neonicotinoids on aquatic invertebrates. The objective of this study was to assess the acute toxicity of six neonicotinoids (imidacloprid, thiamethoxam, acetamiprid, clothianidin, thiacloprid, and dinotefuran) to larval *Hexagenia* spp. (mayfly). Neonicotinoid exposures were conducted in water-only systems for 96 hours, and artificial burrows were added to each beaker to provide a substrate. During range-finding tests, we observed that mayflies exhibited sublethal effects, such as being found outside of artificial burrows and exhibiting reduced swimming activity, at much lower concentrations than those causing lethality. Therefore, both survival and behavioural endpoints, specifically the number of animals inhabiting artificial burrows, were investigated in subsequent definitive tests. Effects of neonicotinoids on mayfly survival were variable among compounds: 96-hour LC_{50}s were 600, 700, and 800 $\mu g \cdot L^{-1}$ for imidacloprid, clothianidin, and acetamiprid, respectively, and were greater than 10,000 $\mu g \cdot L^{-1}$ for thiamethoxam, thiacloprid, and dinotefuran. Mayfly behaviour was affected at much lower concentrations than survival for all

neonicotinoids, with 96-hour EC_{50}s for number of larvae inhabiting artificial burrows of 4, 8, 10, 20, 50, and 200 $\mu g \cdot L^{-1}$ for acetamiprid, thiacloprid, imidacloprid, clothianidin, dinotefuran, and thiamethoxam, respectively. In addition, we observed that mayfly swimming activity was reduced by imidacloprid, acetamiprid, and thiacloprid at 1 $\mu g \cdot L^{-1}$ and higher; further tests to quantify this effect are ongoing. The maximum environmental concentration of neonicotinoids measured in 2012-2014 surveys of 16 stream sites in southern Ontario was 10 $\mu g \cdot L^{-1}$ (imidacloprid), indicating that environmental concentrations may adversely affect sensitive aquatic species.

Relative risk approach: A novel approach for the ecological risk classification of inorganic substances under the next phase of the Chemicals Management Plan (PL)

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[1]Environment and Climate Change Canada

Under the *Canadian Environmental Protection Act, 1999*, Environment and Climate Change Canada and Health Canada assess and manage, where appropriate, risks of chemical substances to the environment and to human health. The Chemicals Management Plan (CMP) is a Government of Canada initiative that addresses approximately 4300 substances identified as priorities for assessment. In the next phase of the CMP (2016-2020), about 1,550 substances remain to be addressed, including approximately 380 inorganic substances. Early activities to address remaining inorganic substances include identifying data needs, developing tailored strategies and novel approaches, and preliminary stages of assessment drafting. In particular, a relative risk approach is under development for identifying and classifying the potential ecological risks of inorganic substances. Predicted and measured environmental concentrations are two key lines of evidence for the relative risk approach for inorganic substances. Data from the Domestic Substances List inventory update (DSL-IU) and National Pollutant Release Inventory (NPRI) combined with conservative exposure scenarios form the basis for determining predicted environmental concentrations. Measured environmental concentrations are determined from surface water quality data from Canadian monitoring and surveillance programs. The relative ecological risk of individual inorganic substances and groups was determined by comparing predicted and measured concentrations to selected predicted no-effect concentrations to determine an overall preliminary risk classification. The relative risk approach is a novel and efficient method allowing Environment and Climate Change Canada to focus ecological assessment efforts on remaining substances of highest ecological concern.

Characterization of the endocrine potencies of municipal effluents across Canada using *in vitro* bioassays (PL)

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Recent years have witnessed increasing concerns regarding the presence of contaminants in the environment that have the potential to adversely affect the endocrine system of humans and wildlife.

Municipal wastewater effluents (MWWEs) are considered one of the major sources for such endocrine-disrupting chemicals (EDCs) in surface waters, as conventional wastewater treatment technologies are frequently inefficient at removing these compounds from raw sewage. However, our understanding of the contribution of MWWEs to environmental endocrine disruption in Canadian surface waters is incomplete. Therefore, the aim of this project was to investigate the EDC removal efficiency of six wastewater treatment plants (WWTPs) across Canada. Specifically, samples of influents and effluents were collected during spring, summer and fall in 2014 and winter in 2015, to evaluate the influence of climatic conditions, season, population size and treatment level (tertiary, secondary, primary) on EDC removal efficiency. Endocrine potentials of wastewater were analyzed using two *in vitro* bioassays: MDAkb2 ((anti-)androgenicity), and MVLN ((anti-)estrogenicity). Preliminary results indicated that most influent samples collected had a significant increase in androgenicity relative to solvent controls, while most effluent samples were less potent and showed variability in response indicating that WWTPs had a high removal efficiency of androgenic activity. Removal efficiencies differed significantly among WWTPs, probably as a function of different levels of treatment. Greater removal efficiency was detected during summer, potentially due to greater metabolic activity, increased temperature and/or light exposure. A select number of influent and effluent samples showed anti-androgen response, suggesting there may be other compounds competing for the same receptor. Most spring effluent samples showed a trend towards elevated estrogenicity, as did fall samples from Regina and Saskatoon. Effluent samples showed higher estrogenic activity than influent samples, and anti-estrogenic trends were detected for the majority of influents and effluents, suggesting samples may contain compounds competing for the same receptor. This project will provide insights into the most effective approach for monitoring MWWEs, and will inform development of advanced wastewater treatment technologies for improved removal of EDCs.

In vitro-in vivo extrapolation of biotransforming hydrophobic chemicals in the fish body (PO)

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A chemical's potential to bioaccumulate in biota is an important factor to consider when assessing its ability to cause harm in organisms. As a result, bioaccumulation is a fundamental criterion in chemical classification schemes under international and national environmental programs (e.g., United Nations Environment Programme, *Canadian Environmental Protection Act, 1999*, U.S. *Toxic Substances Control Act*, European Union's REACH). Under these regulatory programs, the Organisation for Economic Co-operation and Development's (OECD's) bioconcentration factor (BCF) fish Test no. 305 is the preferred assay to evaluate for the bioaccumulative potential of chemicals in the environment. However, these assays are costly (over $125,000 per chemical), require the use of many animals, are difficult to complete and, therefore, very few BCF data exist. Conversely, the less-costly octanol-water partitioning (K_{OW}) test can provide a modest understanding for the bioaccumulative behaviour of chemicals without the use of animals, but does not account for an organism's ability to eliminate a chemical through biotransformation. With no information on biotransformation, this presents a large data gap in bioaccumulation assessments based on octanol-water partitioning and a tendency towards type II errors (i.e., where chemicals are determined as bioaccumulative when they are not). Consequently, there are global objectives to develop new test methods for improving bioaccumulation assessments that are cost-effective, time efficient, while also using fewer animals in testing. *In vitro* biotransformation tests, in combination with *in vitro-in vivo* extrapolation (IVIVE), K_{OW} and bioaccumulation modelling is one initiative to meet these large-scale objectives. This presentation details a method to extrapolate *in vitro*

biotransformation data to *in vivo*. A total of four metabolizable test chemicals—pyrene, 9-methyl anthracene, chrysene, and benzo[a]pyrene—were evaluated both *in vitro* and *in vivo* to determine somatic biotransformation rate constants. The comparison of predicted and observed *in vivo* biotransformation rate constants allows us to assess the performance of the IVIVE method summarized in this study. The overall goal of the study is to improve on the bioaccumulation assessments of commercial chemicals.

Relationships between parasites and plasma proteins in male white sucker (*Catostomus commersonii*) from the Athabasca River (PO)

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Shotgun proteomics can be used to assess the health of animals, to determine protein biomarkers that are specific to environmental exposures, and also to characterize unique mechanisms of action of contaminants. As part of a larger wild fish health assessment for the Athabasca River under the Joint Oil Sands Monitoring program, we successfully developed and applied shotgun proteomics to generate protein profiles from plasma of mature male white sucker taken from three sites along the main stem of the Athabasca River in 2011. The study sites were located within the oil sand deposit including a site downstream of Fort McMurray but above the oil sands operations, and two sites downstream of the oil sands extraction facilities. On average, 376 ± 96 proteins were identified in plasma from each location. Gene names corresponding to those identified proteins were analyzed using interactive pathway software (Ingenuity Systems, Inc.) to determine their core functions and to compare the data sets by location, year, and sex. There were 478 proteins identified in plasma from fish across all sampling locations that were related to immunological functions. The following parasites were enumerated in the same fish samples: *Diplostomum* spp. (eye fluke), *Ichthyocotylurus* sp. (fluke), *Phyllodistomum* sp. (bladder fluke), *Dactylogyrus* sp. (gill flukes), and *Polyopisthocotylea* gen. sp. (flatworm). Linear discriminant analysis (LDA) was performed on the parasite counts for each species and the count of proteins related to various immunological functions. Principal component analysis (PCA) was performed on the relative expression (total intensity counts) of each protein related to immune functioning for all individuals. Both of the multivariate approaches revealed strong differences (based upon protein expression, function, and parasite counts) among the three sampling locations.

Innovative Regulatory Approaches to Assessing and Managing Environmental Effects

Monitoring, reporting, and adaptive management: Connecting the dots between environmental assessment, licensing, and operations for major developments (PL)

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Legal determinations of significant adverse impacts made during environmental impact assessment (EIA) are based on predictions of how a proposed development will affect the environment, and the acceptability of those predicted impacts. If significant adverse impacts are likely, mitigation measures may be applied through the EIA process to reduce the severity of an impact to a level that is acceptable, or below the "significance threshold". To give full effect to and derive the best environmental outcomes from such mitigation measures, monitoring and reporting are needed to: (1) verify that measures are being implemented and evaluate their effectiveness, (2) confirm that significant adverse impacts are not occurring, (3) test environmental assessment predictions, and (4) inform adaptive management. To this end, and with impact predictions and significance thresholds in hand from the EIA process, a robust adaptive management framework or Response Framework can be established to inform operational management of a project. Such frameworks include: (1) an overall framework of action levels or thresholds (which identify when to act); and (2) proposed mitigation options, policies, and practices linked to the action levels (which describe what actions to take). Based on the experience of the Mackenzie Valley Environmental Impact Review Board, this presentation will review the significance spectrum—a process for determining significance—and its relationship with the Response Framework and other tools for monitoring, reporting, and adaptive management that can be used to connect the dots between EIA, licensing, and life-of-project operations. Ultimately, we see adaptive management loops that facilitate learning and continual improvement for: (1) operational management actions, and (2) the environmental assessment process itself and future decision making. In other words, it is not just developers who must respond and adapt to new information, but environmental assessment bodies as well.

Integrating adaptive management into regulatory permits in the Northwest Territories (PL)

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Class A Water Licences for major resource development projects in the Northwest Territories (NT) increasingly require that proponents develop a "Response Framework" to link their Aquatic Effects Monitoring Programs (AEMPs) to an adaptive management process. The need grew out of a lack of consensus among proponents and regulators on how to define and use adaptive management as a response to uncertainty. The Response Framework was developed and implemented as a systematic process to address residual uncertainty from the environmental assessment process; to link predictions from the environmental assessment (EA) to monitoring results and management actions developed as part of the regulatory process; to respond to changes that were not predicted; and, to guide a process of continual improvement in environmental outcomes. The Response Framework assumes that specific

management actions need not be defined *a priori*, but will be determined in response to changes documented by AEMPs. The Framework requires proponents to derive measurable definitions of significant adverse impacts ("significance thresholds") for a project (ideally as part of the EA process) and to translate these to "action levels" during the regulatory and operational phases—pre-defined levels of increasing environmental change that trigger staged management responses such that significant adverse impacts never occur. The EA process contributes to the development of a Response Framework by documenting the predictions of environmental change and what degree of change would be considered significant. With a clear definition of changes to be avoided from the EA, the Response Framework can set action levels and management responses to ensure that such changes do not occur. This approach is both prescriptive and adaptive. It allows timely response without the need to debate the significance of monitoring results or exhaustive *a priori* derivation of adaptive management plans for all possible outcomes. Response Frameworks have been developed for chemical, physical and biological metrics. They are increasingly based on statistically-based deviations from pre-development reference conditions with a resultant need for the development of sound environmental baselines in the AEMP. The principles of the Response Framework will be illustrated with recent examples from NT projects.

Independent Environmental Monitoring Agency (IEMA): An innovative northern initiative (PL)

Marc Casas[1]

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Governments, at all levels, are challenged to find the resources necessary to manage mineral and oil and gas projects. In contrast, environmental concerns resulting from large development projects are increasing. This results in an increased strain on the regulatory system. Non-government independent monitors help support the regulatory system by providing valuable input. Monitors are a board/agency of individuals with technical, scientific or regional expertise that can either directly review and comment on development plans, or can hire the appropriate expertise to do so. Since the mid-1990s in the Northwest Territories (NT), there have been environmental assessments on four diamond mines and one mine reclamation project, which have resulted in the creation of five environmental agreements. The environmental agreements create and outline the purpose, mandate, and funding structure under which the monitors operate. One of the main mandates of the Independent Environmental Monitoring Agency (IEMA) is to ensure that there is a voice to provide independent, defensible, site specific, and technical input on project-related environmental matters at the Ekati Mine into the regulatory system. A second primary function is to communicate with our Aboriginal society members (four Aboriginal organizations and governments) regarding questions they have about the mine, and to raise issues they have concerning the mine with Dominion Diamond Ekati Corporation, the owner/operator. The regulatory system can only make decisions based on the information available to it. A reduction in quality and quantity of reviewer input weakens the regulatory system's ability to ensure proper environmental management. Government budget cuts, downsizing and restructuring are making it increasingly difficult for government agencies to keep up with the demands of environmental regulation in the NT. Monitors have proven to be a critical component of the regulatory system. The four diamond mines that have monitors in the NT boast some of the most rigorous environmental management plans and monitoring programs in Canada. This is no coincidence. The valuable input provided by the monitors over the years has been essential in ensuring that developers maintain this high quality of work. Perhaps it is time for other jurisdictions to look north and consider the value of monitors.

Advantages associated with two different approaches to regulatory oversight (PL)

Alexandra Hood[1]

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De Beers Canada Inc. owns two mines in the Northwest Territories (NT): the Snap Lake Diamond Mine, which is currently on Care and Maintenance, located 220 km northeast of Yellowknife, NT, and the Gahcho Kué Project, a joint venture between De Beers and Mountain Province Diamonds that is currently in construction, located approximately 280 km northeast of Yellowknife. The environmental monitoring of these two mines features arm's-length review, undertaken in different ways. This talk will discuss the advantages, from a proponent's perspective, associated with the two different approaches to regulatory oversight. In the first approach, the Snap Lake Environmental Monitoring Agency (SLEMA) was established as part of an environmental agreement between De Beers Mining Canada, the Government of Canada, the Government of the Northwest Territories and a number of local Aboriginal groups. The main purpose of SLEMA is to act as a public oversight organization to ensure environmental regulatory compliance and to ensure appropriate and comprehensive inspection processes by government regulators. The Agency's Board is comprised of eight representatives from the four signatory Aboriginal groups. The Board strives to involve Aboriginal traditional knowledge and conventional science in its assessment of mining activities and environmental reports submitted by De Beers and government inspectors. To achieve this goal, the Agency has both a traditional knowledge panel made up of elders that have hunted, trapped and lived in the area of the mine site, and a science panel made up of experts who are foremost in their field and familiar with working in the Northwest Territories. For the second approach, taken at the Gahcho Kué Project, environmental monitoring oversight is undertaken by Ni Hadi Xa (NHX), a collaborative organization formed by five signatory local Aboriginal communities. While similar in composition and purpose to SLEMA, NHX employs slightly different mechanisms to ensure that impacts associated with Gahcho Kué are avoided or minimized. Decisions are made by a Governance Committee, comprised of one member from each of the Parties, including a company representative (NHX features a cooperative approach where the company is an active member). Three streams of monitoring are undertaken by NHX: (1) observational, where there is an environmental monitor at site 50% of the time; (2) technical, where an experienced staff member provides reviews of regularity documents, plans and programs; and, (3) traditional knowledge, where two dedicated monitors will work with families who travel to areas around the mine to hunt, fish and trap—living life as they would normally. It is this last step which is novel—using the experiences of land users and families as one of the primary mechanisms for observing the environment.

Developing a load management approach for water quality in the North Saskatchewan River (PL)

Alyssa Tuininga[1] *and Mary Joy Wesley*[1]

[1]*Alberta Environment and Parks*

The Water Management Framework for the Industrial Heartland and Capital Region is a world-class integrated water management system to sustainably support the environment, social and economic development within the North Saskatchewan River watershed in Alberta, Canada. This Framework was released in 2007 as part of Alberta's new Cumulative Effects Management Framework to address the proposed growth and development within the region. The overall intent of the Framework is to better manage water quality and quantity with the main outcome of the Framework to “maintain or improve

water quality". Implementing this Framework has included stakeholder engagement as a fundamental component in delivery of the Framework. Initial work included establishing water quality baseline conditions and building on regional scientific knowledge. Recently, a load management approach was identified as the management process to follow to meet the Framework goal of "maintain or improve water quality". This load management approach is being implemented using a phased approach. This approach started with the development of maximum allowable loads. Loosely based on the Total Maximum Daily Load approach used in the United States, maximum allowable loads for variables of concern were developed at both Devon and Pakan to determine what seasonal mass of pollutants the river could sustain without seeing a decrease in overall water quality. To understand sources of the majority of the pollutants, the Capital Region is implementing an Effluent Characterization Program for direct industrial dischargers to the North Saskatchewan River within this Devon to Pakan reach. Concurrently with this program, policies, operating practices and regulatory processes are evolving to ensure that water quality loads can be placed in regulatory approvals to make certain water quality in the North Saskatchewan River is being maintained. This load management approach is part of the first application of cumulative effects management in the province, resulting in innovative approaches when managing water quality. This presentation will touch on the load management process and the main components of this process, the maximum allowable loads, and the effluent characterization program. Attendees will hear about the development of the effluent characterization program with a specific focus on the learnings to date and the current implementation of the program.

Towards an integrated water management approach for oil sands mines (PL)

Kim Westcott[1], Sarah Moody[1], Kusumakar Sharma[1] and Thorsten Hebben[1]

[1]Alberta Environment and Parks

On March 13, 2015, the Government of Alberta released the Lower Athabasca Region: Tailings Management Framework for Mineable Athabasca Oil Sands (TMF). The main objective of the TMF is to minimize fluid tailings accumulation by ensuring that fluid tailings are treated and reclaimed during the life of a project. The TMF acknowledges that as oil sands mines accelerate the treatment of fluid tailings, more water will be liberated from the tailings matrix. Over time there will be increasing pressure to effectively manage this water, in addition to the large inventories of oil sands process-affected water some mine operators are already managing. A new approach to water management is needed to assist oil sands mine operators in making good water management decisions as they move forward with their tailings and reclamation goals. To change how water is currently managed on oil sands mine sites, water management activities will need to be assessed more comprehensively and be better integrated (i.e., supply, use and disposal). The full range of water management options will be available to mine operators, including the treatment and release of water generated through tailings treatment and bitumen extraction. This will be accompanied by enhanced regulatory oversight and new expectations for environmental performance. The purpose of this talk is to provide an overview of the key elements and principles of this new water management approach, including discussion of emerging requirements and key areas for future work.

Turbidity monitoring: An improved approach to quantifying in-stream construction impacts (PL)

Greg Courtice[1]

[1]*Amec Foster Wheeler*

Existing turbidity monitoring requirements are based on generalized toxicity guidelines, which do not address short- to medium-term (i.e., 1-15 days) irregular activities, which are commonplace for infrastructure works around watercourses. In many cases, these regulatory requirements result in undue impacts to the aquatic environment and increased project costs. Amec Foster Wheeler has developed an alternative turbidity monitoring approach to determine real-time aquatic impacts due to heightened sediment loading during construction activities. This approach gives a more accurate representation of construction impacts, leading to greater construction flexibility, reduced costs, and reduced environmental impact during construction. This approach may provide economic and environmental benefits to in-stream construction projects such as flood and erosion control, pipeline crossings, bridges/culverts, outfalls, etc.

Updates to the proposed Canadian regulatory framework for the environmental assessment of new active ingredients in human and veterinary drugs (PL)

Tariq Francis[1], Julie Chateauvert[1] and Sigrun Kullik[2]

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A Canadian regulatory framework has been developed specifically for active ingredients in human and veterinary drugs regulated by the *Food and Drugs Act* (F&DA) to assess risks to the environment and to human health resulting from environmental exposure. This regulatory framework has been designed to harmonize with the drug approval process stipulated by the F&DA and its regulations. Health Canada developed this framework in collaboration with stakeholders, including Environment and Climate Change Canada, industry, and environmental non-government organizations. The framework was endorsed in principle by all stakeholders in 2011. Since then, the proposed regulatory framework has been revised to increase alignment with environmental assessment approaches in other jurisdictions and to incorporate recent technical developments related to the environmental assessment of active ingredients in human and veterinary drugs. Highlights include a proposal to adopt VICH (International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products) guidelines 6 and 38 for environmental assessment of active ingredients in veterinary drugs, and updates to screening level exposure assessments, such as new Canada-specific defaults for predicting environmental concentrations of veterinary drugs in soils. The proposed changes will be the subject of an upcoming stakeholder consultation. The purpose of this presentation is to present the revised regulatory framework being proposed and generate feedback on the updates.

Review of Ontario municipal-industrial strategy for abatement chemical and aquatic toxicity data and sector analysis (PO)

Andrea Farwell[1], Che Lu[1], Dong Zhang[2], David Poirier[2], Sonya Kleywegt[2], Tim Fletcher[2] and D. George Dixon[1]

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In 1987, the provincial Municipal-Industrial Strategy for Abatement (MISA) under the Ontario *Environmental Protection Act* was established to reduce contaminants entering Ontario waters via discharge of industrial wastewater. From 1988 to 1993, initial monitoring that consisted of a comprehensive list of chemical and physical parameters, as well as acute toxicity to rainbow trout (*Oncorhynchus mykiss*) and *Daphnia magna*, was established for nine industrial sectors. Following a review of the initial monitoring data and on the basis of reduction of toxicity and Best Available Technology Economically Achievable (BATEA), the Effluent Monitoring and Effluent Limits (EMEL) regulations were promulgated. Since the establishment of EMEL regulations, no thorough review of the monitoring data has been conducted. The objective of this study is to analyze available toxicity test results and associated water quality measurements to assess the effectiveness of the EMEL regulations in reducing toxicity to standard test species. Rainbow trout and *D. magna* toxicity tests results from 1995 to 2015 were provided for nine sectors (electric power generation, industrial minerals, inorganic chemicals, iron and steel manufacturing, metal casting, metal mining, organic chemical manufacturing, petroleum refineries, and pulp and paper). All toxicity tests results were reported as “pass” and “fail”. A “fail” under the EMEL monitoring regulations indicates that over 50% of the test animals died in the 100% (undiluted) effluent sample being tested. For each toxicity test, results were compiled by sector, company (numerical code only) and year. Over 36,000 results for rainbow trout and *D. magna* toxicity tests were reviewed and analyzed. A total of 203 companies from nine sectors were identified; 75-100% of the companies reported toxicity test results. The total number of test results varied by sector with less than 200 toxicity test results for metal casting and greater than 10,000 toxicity test results for pulp and paper. Across sectors, between 30% and 90% of companies reported one or more “fails”, representing a total of more than 1,500 failed tests. Lethal toxicity test “fails” will be correlated with available water chemistry monitoring data to assess the effectiveness of these monitoring components of the EMEL regulations.

Expecting the Unexpected: Case Studies in Responding to Spills and Unintentional Releases

A weight-of-evidence study assessing the potential for toxic effects on sensitive aquatic habitat following a coal train derailment in an urban environment (PL)

Guy Gilron[1], Jennifer Trowell[2], Karla Graf[3], Luanne Patterson[3], Francisco Perello[2], Christina Chan[2] and Shannon Bard[4]

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There is a general paucity of information regarding the environmental impact of accidental spills of raw, washed coal in freshwater ecosystems. The January 2014 derailment of three coal-containing rail cars in Burnaby, British Columbia, resulted in coal being deposited throughout Silver Creek and Burnaby Lake, including an area located immediately offshore of nesting locations of western painted turtle (*Chrysemys picta bellii*) and other species. By May 2014, remedial efforts in both waterbodies resulted in >90% removal (by volume) of the accessible spilled coal. The remediation undertaken at and adjacent to Turtle Beach involved the salvage of juveniles and coal removal immediately offshore of the nesting beach at the confluence of Silver Creek and Burnaby Lake. In order to evaluate residual impacts from any unrecovered coal downstream of the spill area and associated temporal effects, aquatic impact assessments of the receiving environment were conducted in the spring of 2014 and 2015. The assessments focused on potential short- and long-term water and sediment quality impacts, using a weight-of-evidence approach. Study elements included evaluations of: chemical tracers of the spilled coal, water and sediment quality, sediment/porewater toxicity, bioaccumulation potential of sediment contaminants, and direct measures of remaining coal. Concentrations of constituents measured in water were below applicable aquatic life guidelines. Concentrations of various metals and polycyclic aromatic hydrocarbons (PAHs) measured in sediment collected just downstream of the recovery area exceeded both sediment quality guidelines and concentrations in reference areas. Relative concentrations of low versus high molecular weight PAHs in downstream sediments were consistent with those measured in metallurgical coal, implying that coal was present in downstream sediments. Evaluation of aquatic invertebrate toxicity/bioaccumulation test data provided more specific information regarding bioavailability and potential for biological impact and temporal improvements, post-remediation. For both years and all locations—except for one station—site sediments/porewater were non-toxic to the species tested. Although this latter station yielded toxicity to midge and amphipod survival in 2014, no toxicity was observed in 2015. Similarly, although 2014 bioaccumulation potential test results for freshwater oligochaetes indicated that sediment PAHs downstream of the derailment site had slight potential to bioaccumulate, the same was not evident in 2015. These results suggest that the remediation efforts were successful in sufficiently reducing coal in sediment to concentrations posing a low potential for adverse impacts to aquatic receptors. Based on these results, it was recommended that sediments be left in place for the residual coal to attenuate naturally, as any further sediment removal would likely pose greater risks to aquatic receptors through habitat disturbance and resuspension and transport of residual coal particles over a broader area.

Assessing the ecotoxicity of crude oils, chemical composition, exposure time and importance of biomarkers (PL)

Ramin Memarian[1] and Heather D. Dettman[1]

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From the viewpoint of ecotoxicity, crude oils are of most concern because they contain complex chemical compositions of hydrocarbons and non-hydrocarbons. When spilled in the ecosystems, multiple fate and transport pathways occur; each has a different toxicity level to the biota and to the living environment, which could further create secondary impact in terms of bioaccumulation. Basically, the uniqueness of the toxic oil product problem lies in the potential transfer of specific hydrocarbons, BTEX (benzene, toluene, ethylbenzene, and xylenes), to organisms through two principal routes: ingestion from the water and from contaminated aquatic foodstuffs. However, exposure to larger hydrocarbons such as polycyclic aromatic hydrocarbons (PAHs) may not always result in significant harm to organisms immediately. Nevertheless, those are important agents in causing chronic effects when present over long periods of time. Advances in characterizing crude oils have provided more information on chemical composition, which needs to be considered for the assessment of ecotoxicology results, with a focus on biomarkers. The log K_{OW} (octanol-water partition coefficient) values presented in the study by Di Toro et al. (2007) are taken to build a hierarchical model of PAHs to reveal the similarities in terms of bioconcentration factors. Toxic units (TUs) are applied to reflect acute toxic potential of PAHs for fish, in accordance with the acute lethality model suggested by McCarty (1991). The TUs model will be applied to compare the difference between selected PAHs of fresh and weathered diluted bitumen and conventional crude.

Di Toro D.M., McGrath J.A., and Stubblefield W.A. 2007. Predicting the toxicity of neat and weathered crude oil. Toxic potential and the toxicity of saturated mixtures. *Environmental Toxicology and Chemistry*, 26(1): 24-36.

McCarty, L.S. 1991. Toxicant body residues: implications for aquatic bioassays with some organic chemicals. *In:* Mayer, M.A., Barron, M.G. (Eds.), Aquatic Toxicology and Risk Assessment, ASTM STP1124, vol. 14. American Society of Testing Materials, Philadelphia, pp. 183-192.

Environmental impacts of the largest coal slurry spill in North America: The Obed Mountain Mine release into the Athabasca River (Alberta, Canada) (PL)

Colin Cooke[1], Colin Schwindt[2], Martin Davies[2], William Donahue[1] and Ekram Azim[2]

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On October 31, 2013, a catastrophic release of approximately 670,000 m^3 of coal process water occurred as the result of the failure of the wall of a post-processing settling pond at the Obed Mountain Mine near Hinton, Alberta. A highly-turbid plume entered the headwaters of the Athabasca River approximately 20 km from the mine, markedly altering the chemical composition of the Athabasca River as it flowed downstream. Over the next four weeks, the released plume traveled approximately 1,100 km downstream eventually reaching Peace-Athabasca Delta (a Ramsar Wetland of International Significance located within Wood Buffalo National Park) and Lake Athabasca. The plume itself was tracked both visually and using real-time measures of river water turbidity within the Athabasca River. The plume initially contained high concentrations of nutrients (nitrogen and phosphorus), metals, and polycyclic aromatic hydrocarbons (PAHs); some Canadian Council of Ministers of the Environment

(CCME) guidelines were exceeded in the initial days after the spill. Aerial imagery assessment was used to identify 487 potential sediment depositional areas along the mainstem of the Athabasca River. A subset of these areas was surveyed in May 2014, and material from the release material was easily identified visually. Samples were collected for geochemical analysis and compared to source material from the mine. The released material contained elevated concentrations of both metals (arsenic, lead, mercury, selenium, and zinc) and PAHs (acenaphthene, fluorene, naphthalene, phenanthrene, and pyrene). The spill has the potential to exert negative long-term impacts especially in impacted areas closest to the mine.

Higher than expected metals and pesticide concentrations from major tributaries and approved wastewater effluents into receiving mainstem rivers: A southern Alberta synoptic survey case study (PL)

Natalie Kromrey[1]

[1]*Alberta Environment and Parks*

During August 12-22, 2014, 155 water samples were taken from mainstem rivers, major tributaries and wastewater facilities (that continuously discharge to a mainstem river) in the South Saskatchewan River basin of southern Alberta, Canada. Samples were taken in an upstream to downstream fashion from the headwaters of the Red Deer, Bow and Oldman rivers to their eventual confluence in the South Saskatchewan River as a hydrologically calculated time of travel (synoptic) survey. Samples were analyzed for physical and biological parameters, nutrients, bacteria, pesticides and total and dissolved metals. Higher than expected results were found for metals (total: mercury, methylmercury, arsenic, copper, molybdenum, selenium etc.) and pesticides (2,4-D, dicamba, MCPA, MCPP and 13 other pesticides) in wastewater effluents, while higher than expected metals results were found in particular tributaries (total: mercury, arsenic, cadmium, cobalt, copper, lead, manganese, nickel, selenium, silver, thallium, uranium, zinc, etc.). Further investigations successfully determined (and rectified) the source of high mercury and metals for one facility while the high frequency of pesticide detections in multiple wastewater effluents has yet to be fully understood. Additionally, high tributary metal concentrations sparked a reassessment of certain fish consumption limits, but the contributing cause has yet to be identified. The nature of these parameters—how often they are present in continuously discharged effluent and major tributaries, and at what concentrations—is currently not adequately characterized. More work is needed to understand the cumulative impact on the receiving water environment. These unexpected results support this style of monitoring and stimulate reflection on requiring all facilities to monitor for unpredicted parameters as part of their operating approval. Moreover, sampling for the unexpected in the ambient environment and being surprised is just as important as only sampling to find answers to predetermined questions.

Genomics in Ecotoxicology

Application of genomics as indicators of freshwater wildlife health (PL)

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[1]University of Victoria, [2]British Columbia Cancer Agency Genome Sciences Centre, [3]Environment and Climate Change Canada

Frogs are harbingers of environmental status and surrogates for human health. The Ranidae, or "true frogs", comprise the largest anuran family and its representatives span the globe. In an effort to effectively utilize gene expression as indicators of adverse outcomes, we have been actively generating molecular resources for amphibian species. We have generated a good draft of the first "true frog" genome from *Rana (Lithobates) catesbeiana* and compare this to the genomes representing common laboratory frogs of the Pipidae family. Bullfrog tadpoles were exposed to thyroid hormones, estrogen, or a cocktail containing a mixture of pharmaceuticals and personal care products that are commonly found in municipal wastewater. RNA-seq and qPCR analyses were performed on select target tissues using information gained from our genome cross-validated with *de novo* assembled transcripts. Changes in biomolecule abundance and their linkage to adverse developmental outcomes will be presented. *De novo* assembly of transcripts proved to be an invaluable and economical source of information for genome validation, but is also a significant enabler for species that do not have an available genome. Both *de novo* transcript and genome assembly pipelines can be readily applied to any species with a complex genome.

Reconstructing cyanobacterial population trends in freshwater lakes using metagenomic techniques and physicochemical analyses (PL)

Timothy Tse[1], Song Tang[1], Steve Wiseman[1], Lorne Doig[1], Markus Hecker[1], John Giesy[1] and Paul Jones[1]

[1]University of Saskatchewan

Water quality degradation caused by increased nutrient pollution is a global environmental issue affecting freshwater lakes and reservoirs. Addition of limiting nutrients often leads to eutrophication and the production of harmful algal blooms. This has resulted in significant water quality issues for many potable waters in North America. However, long-term monitoring data are often lacking for many of these sources of water and studies are frequently initiated only after serious problems arise. Natural conditions of aquatic systems or their long-term trajectories are often unknown. Paleolimnological techniques can reconstruct long-term environmental trends in inland waters when monitoring data are limited or unavailable. An emerging tool in paleolimnological investigations is metagenomic analysis, the analysis of genetic material extracted from environmental samples. In this study, sediment cores were collected from a freshwater prairie reservoir, and a combination of physicochemical and metagenomic analyses were conducted to investigate trends and identify relationships among the algal and cyanobacterial communities and various physicochemical variables. Temporal trends in algal compositions of communities were identified through analyses of phytopigments in cores of sediments. Insight into trends in the cyanobacterial community was gained through 16S rRNA sequencing. Finally, a component for the microcystin toxin-producing pathway, the mcyA gene, was identified in sections of sediment cores and was positively correlated to the abundance of Dolichospermum, a genus of harmful

cyanobacteria commonly found in affected freshwater lakes and reservoirs. Metagenomic analyses, in combination with supporting physicochemical characteristics, can provide insight into long-term lake ontogeny and serve as a practical proxy for long-term monitoring data where such data are lacking.

The identification of fish cell biomarkers to detect environmental genotoxic stress (PO)

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In eukaryotes, histones play a crucial role in guarding the integrity of the genome. During situations of external factors causing genotoxic stress such as double-stranded breaks (DSBs) and stalled replication forks, a variant known as H2AX is recruited to chromatin and is phosphorylated at its C'-terminal SQE site by PI3K-like kinases such as ataxia telangiectasia mutated (ATM), ATM- and Rad3-related protein (ATR), and DNA-dependent protein kinase catalytic subunit (DNA-PKcs) in order to rapidly alter chromatin structure and initiate a critical signalling cascade required for resection, repair, and/or apoptosis. Though phosphorylated H2AX (γH2AX) is dispensable for initial recognition of DSB sites, it is responsible for the greatly accelerated recruitment of repair factors to damaged foci, and is an early signature of severe genotoxic stress in cells. An important downstream factor of γH2AX in the DNA damage response (DDR) is the tumor-suppressor protein p53, an effector protein that regulates processes including DNA repair, cell-cycle arrest, senescence, and apoptosis. During the DDR, p53 can be activated via phosphorylation by ATM, ATR, and by key signal transducers Chk1 and Chk2. Once active, p53 is able to transcriptionally regulate many other genes involved in these processes. Compared to mammals, p53 function has been shown to differ in fish and other lower vertebrates, where known genotoxic stimuli fail to induce expressional changes in p53, suggesting that the mechanism of DNA repair in fish may differ from well-studied mammalian models. In the present study, various brain and intestinal myofibroblast cell lines from teleost fish species (Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), and lake sturgeon (*Acipenser fulvescens*)) were treated with model DNA-damaging agents bleocin and methyl methanesulfonate (MMS) in order to profile dose- and time-dependent expression of both γH2AX and p53. We found that the activation of γH2AX is conserved between mammals and the studied species in response to genotoxic stress, and that sensitivity differs between similar cell types across different species. In addition, we observe that p53 expression differs between our studied teleosts, and in sturgeon and salmon it may have a reduced role in the DDR compared to trout. This study highlights the potential of γH2AX and p53 as specific biomarkers of genotoxicity, as well as candidate cell lines to assay the status of environmentally affected waters.

Thyroid hormone disruption in aquatic systems affects metamorphosing tadpoles' ability to perform critical tasks such as locating food and avoiding predation (PO)

Kevin Jackman[1], Nik Veldhoen[1], Rachel Miliano[2], Linda Li[3], Xiaoyu Zheng[4], Azadeh Khojasteh[1], Eric Hall[4], Wayne Parker[3], Graham van Aggelen[2] and Caren Helbing[1]

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Olfaction is a critical component of tadpole survival, providing the ability to locate food and to avoid predators. Olfaction requirements change during amphibian metamorphosis as an herbivorous tadpole transitions from aquatic to terrestrial environments and becomes a carnivorous frog. Little is known

about the influence of thyroid hormones or endocrine-disrupting compounds (EDCs) found in household and industrial products on the olfactory system. The American bullfrog, *Rana (Lithobates) catesbeiana*, is a model organism for studying thyroid hormone action in vertebrate species. Metamorphosis from an aquatic tadpole to a terrestrial frog involves reconstruction of nearly every tissue in the amphibian's body and is entirely dependent on the action of thyroid hormones, L-thyroxine (T4) and 3,5,3'-triiodothyronine (T3). Premetamorphic *R. catesbeiana* tadpoles lack any measurable circulating thyroid hormone and exogenous TH exposure results in premature metamorphosis. In the present study, the olfactory impact of exposure to various hormones and known EDCs was investigated. Premetamorphic tadpoles were separately exposed to environmentally-and physiologically-relevant concentrations of T3, T4, 17β-estradiol (E2), and multiple concentrations of a chemical cocktail of known EDCs typically found in municipal wastewater. In a separate experiment, municipal wastewater was spiked with this chemical cocktail and subjected to either anaerobic membrane bioreactor (AnMBR) or membrane enhanced biological phosphorus removal (MEBPR) processes. After 48 hours of exposure, the olfactory bulb from the brain and the olfactory epithelium from the rostrum were dissected from tadpoles. RNA was extracted from the tissues and transcript levels were evaluated using RNA sequencing and targeted qPCR. Gene expression data indicate that both tissues are responsive to thyroid hormones. A collaborative study currently being conducted to monitor behavioural endpoints will lend insight into the physiological correlations of these molecular findings. In linking biomolecules to behaviour, we hope to elucidate important aspects of thyroid hormone-dependent processes in the olfactory system and the effects of disruption by anthropogenic EDCs.

Endocrine-disrupting effects on innate immunity in *Rana (Lithobates) catesbeiana* tadpoles (PO)

Shireen Partovi[1], Nik Veldhoen[1], Rachel Miliano[2], Linda Li[3], Xiaoyu Zheng[4], Azadeh Khojasteh[4], Eric Hall[4], Wayne Parker[3], Graham van Aggelen[2] and Caren Helbing[1]

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Thyroid hormone (TH) facilitates a number of crucial processes in vertebrates, including growth, metabolism, and development. In frog tadpoles, TH is responsible for the metamorphosis of aquatic larvae into terrestrial frogs. During this process, the body of the animal undergoes extensive internal and external changes in preparation for a terrestrial life. The innate immune system, in particular, is significantly altered by TH modulation during metamorphosis, and is fundamental to the survival of the tadpole during this energetically demanding process. Tadpole liver and tail fin are heavily involved in the innate immune response and are valuable organs to study during metamorphosis as well as during disease challenge. Disruptions to vital TH pathways have negative implications for the intricate process of tadpole development, which presents the opportunity to use frogs as sentinels for research on TH disruption. Endocrine-disrupting chemicals (EDCs) interfere with hormone signalling in vertebrates, including amphibians. These include chemicals from sources such as pharmaceuticals, herbicides, pesticides, and personal care products. Conventional wastewater treatment methods do not fully remove EDCs, allowing low concentrations to persist in treated wastewater effluent. These low-level EDCs may remain biologically active and disruptive to the endocrine system of developing tadpoles. TH pathway disruption that affects the development of innate immunity in tadpoles may result in increased susceptibility to disease and infection. In the present study, we exposed American bullfrog (*Rana (Lithobates) catesbeiana*) pre-metamorphic tadpoles to the THs 3,5,3'-triiodothyronine (T3) and thyroxine (T4), the sex-hormone 17β-estradiol (E2), as a non-TH control, a cocktail of known EDCs, and

treated municipal wastewater effluent. Gene expression was evaluated in the tail fin and liver using RNA-seq and targeted qPCR assays to determine the impact of these chemicals on various genes and pathways, including those relating to the innate immune system. It has been empirically demonstrated that the innate immune system of bullfrog tadpoles is affected by exposure to TH. The presented work will further our understanding of the connection between EDCs and innate immune system perturbation.

Wildlife Toxicology and Population Implications

Polycyclic aromatic hydrocarbon impacts on pre-migratory fuelling in two shorebird species (PL)

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Many shorebirds are currently failing to sufficiently fuel prior to departure for migration, which may be contributing to population declines in these birds. Proper fuelling is important because it increases a bird's probability of surviving migration and it determines migration speeds, which are correlated with reproductive performance. The polycyclic aromatic hydrocarbons (PAHs) found in oil pollution have the potential to interfere with avian pre-migratory fuelling physiology. However, a link between PAH exposure and impaired pre-migratory fuelling has yet to be established. Our objective was to determine whether PAH contamination affects pre-migratory fuelling in two shorebird species. We captured over 35 red knots (*Calidris canutus*) and over 375 sanderling (*Calidris alba*) from Chaplin Lake, Saskatchewan, a relatively uncontaminated site, and from the Gulf of Mexico, which is subject to recurring oil spills. We determined plasma PAH levels using an immunoassay, body condition using mass and fat score measurements, and fuelling status using plasma metabolite levels. We also used Motus radio telemetry array technology to track the arrival, departure, and stopover duration of over 20 red knots and over 75 sanderling. We found that birds in the Gulf of Mexico had longer minimum stopover durations, which were associated with body condition and fuelling status at capture. Using a combination of captive dosing and field studies at multiple sites, we are currently testing the hypothesis that higher plasma PAH concentrations are associated with altered plasma metabolite profiles, body conditions, and stopover durations. This work will inform shorebird conservation by providing valuable insight into a potential cause of long distance migratory shorebird declines.

Biomarkers and ecological tracers provide useful information in support of monitoring contaminants in great blue heron eggs and nestlings along the St. Lawrence River (PL)

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The great blue heron (*Ardea herodias*; GBHE) nests in many colonies along the St. Lawrence River, in freshwater, estuarine and marine habitats. Since 1991, GBHE eggs were collected as part of an environmental monitoring program in colonies distributed in five regions: inland, fluvial, upper estuary, lower estuary and Gulf, and analyzed for mercury (Hg), polychlorinated biphenyls (PCBs), persistent organic contaminants (OCs) and brominated diphenyl ethers (BDEs), as well as stable isotopes δ13C and δ15N. In addition, contaminants (OCs, PCBs, BDEs and Hg) were measured in the blood of GBHE nestlings and Hg was analyzed in feathers. The effects of long-term exposure to persistent contaminants were monitored with biomarkers known to be essential for growth, development and reproduction: retinoid (vitamin A) and thyroid hormones (T3 and T4). In eggs, most contaminants declined significantly over time in most regions. Higher levels of Hg, PCBs, OCs and PBDEs were observed in freshwater colonies than in estuarine colonies, both in eggs and in nestlings. Levels of BDEs in eggs remained high in the fluvial region until 2001 and declined thereafter. GBHE eggs showed a large range in δ13C and δ15N

values, reflecting the broad feeding strategies of wading birds. Fluvial and inland regions showed the lowest values for δ13C and δ15N, while the Gulf exhibited the highest values. δ13C showed a significant decrease over time in the fluvial region and a significant increase in the Gulf region; these results indicate that, with time, birds from fluvial colonies became progressively associated with a carbon depleted diet indicative of an aquatic food web, while birds from Gulf colonies were foraging on more inshore prey items than in the past. δ15N showed a significant decrease in the fluvial region and a significant increase in the upper estuary; this indicates that, in fluvial colonies, birds tended to feed at a lower trophic level while herons from the upper estuary colonies feed at a higher trophic level than in the past. In the freshwater colonies, both δ13C and δ15N showed significant correlations with PCBs and BDEs, but not in the estuarine and marine colonies. Concentrations of contaminants in heron nestlings were generally below critical thresholds reported for adverse effects observed on reproduction or survival. Levels of retinol, dehydroretinol (DROH), thyroxine (T4) and triiodothyronine (T3) were generally higher in estuarine colonies than in freshwater colonies. Retinol concentrations were negatively related to PCBs, while DROH concentrations were negatively related to Hg and total and free T3 concentrations were negatively related to PBDEs. Although our observations of number of nests and nestlings indicated stable populations, the GBHE nestlings from freshwater colonies, exposed to relatively high concentrations of a large cocktail of contaminants, had reduced levels of retinol and thyroid hormones, which could impair their development and fitness.

Population genetic tools for non-invasive monitoring of North American river otter population dynamics (PL)

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Genetic monitoring of wildlife populations is increasingly applied to assess wildlife population health and temporal changes in population dynamics to aid protection of biodiversity. In this context, non-invasively collected fecal samples for genetic analysis have become an important monitoring tool in wildlife management, population and conservation genetics, and phylogeography. At the same time, fecal samples provide other essential information such as contaminant burdens, hormone level responses, and intestinal parasite loads, and therefore, fecal genotyping presents a significant step linking genetic data to other wildlife monitoring results. This may be especially true for elusive species like the North American river otter (*Lontra canadensis*), for which population size estimates and migration rates have been difficult to obtain. Hence, the optimization of fecal genotyping protocols holds the promise to identify unique individuals (i.e., repeated collections of the same animals can be identified) to establish a diverse range of population genetic diversity estimates in order to study impacts of, for example, contaminated habitats on population dynamics. However, genetic amplification success rates from river otter scats have been low (12-31%) and therefore, hamper non-invasive monitoring efforts in river otters. Here, we present a refined method of non-invasively collecting fecal samples in the field for North American river otters which is cost-efficient (i.e., no shipping on ice or liquid nitrogen) and fairly easy to implement; therefore, the method might be applicable for wide-ranging Indigenous community-based monitoring programs or citizen science initiatives. Finally, we discuss how population genetic tools may aid monitoring efforts in this and other species.

Evaluating mercury guidelines for furbearers using a predictive meta-model (PL)

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Abstract

Current screening guidelines for mercury body burden level in furbearing mammals were set in the 1990s with little revision or evaluation since. Since guideline development, mercury data have been generated on dose-effect relationships for furbearers, particularly river otter (*Lontra canadensis*) and mink (*Neovision vision*). We developed a meta-regression model combining sample averages of diet, fur, brain, liver, kidney, and muscle mercury tissue concentrations using data from peer-reviewed literature. Data from over 6000 samples, pooled across 16 studies, and 96 sampling sites in North America and Europe were used to create the weighted regressions, individually regressing each mean tissue concentration against another. The models selected to represent each pathway were chosen based on a balance of meeting weighted least square assumption of residual homoscedasticity, model fit (AICc), and parsimony for ease of use and interpretation of the conversion factor. The regressions' beta coefficients were used to create the compressive meta-regression; 16 regressions were derived for each river otter and mink. All regressions were statistically significant at a 95% confidence interval and on average could explain 85% of the variance. The models were also validated using an external data set of individual mercury tissue concentrations generated through other monitoring programs. Using this model to evaluate the current 30 $\mu g \cdot g^{-1}$ and the more conservative 20 $\mu g \cdot g^{-1}$ fur mercury screening guideline indicates that 30 $\mu g \cdot g^{-1}$ is not conservative enough. At this fur concentration level, the estimated brain mercury concentrations surpass values shown to alter brain neurochemistry. This model can also be used to derive conversion factors for mercury concentrations in fur versus internal organs, thus eliminating the need to collect invasive tissues (e.g., brain tissue) for future monitoring programs.

Introduction

Mercury is a global pollutant of environmental health concern. The element is released to the environment through natural sources, such as volcanoes and forest fires, as well as anthropogenic sources, such as industrial processing and refining of petroleum products amongst others. Once in the environment, mercury can be methylated through microbial activity, thereby increasing its bioavailability. The methylated mercury (MeHg) can be readily bioaccumulated and biomagnified along the food chain. As a result, top predators including fish, river otter, mink, and humans are at the greatest risk of mercury exposure and poisoning (Driscoll et al. 2013). The health effects of mercury are well established, including neurological and cardiovascular effects (Mergler et al. 2007). MeHg can also cross the placenta, making the developing fetus most susceptible to long-term neurological deficits (Mergler et al. 2007).

River otter and mink are common bioindicator species in an aquatic ecosystem as they are piscivorous (top predators), have relatively small home ranges, are exposed to mercury all year long as they do not migrate nor hibernate, and they are widely distributed throughout North America. Further, these species can be used as sentinels for human health, providing advanced warning of mercury levels in an environment and trophic niches shared with humans.

Within these sentinel species, biomarkers are often measured to monitor the level of mercury exposure and the associated health effects (Basu et al. 2007). Common biomarkers include mercury concentrations in fur, brain, kidney, liver, and muscle. The relationship between mercury concentrations in different tissues is not clear, making comparison between different studies difficult. Additionally,

current screening guidelines for mercury body burden levels in furbearing mammals were set in the 1990s and based on limited evidence.

Methods

The objective of this study was to construct a predictive meta-regression model to describe the relationship between the concentration of mercury in each compartment (fur, brain, kidney, liver, muscle) using mean mercury tissue concentrations extracted from peer-reviewed literature on river otter and mink. We reviewed results from 96 sampling sites in North America and Europe reported in 16 papers published from 1982 to 2016 and compiled a data set with a total of 6000 samples. Weighted regression models were created by individually regressing each mean tissue concentration against another, where the weight was set as one over the squared standard error of the dependent variable. All models were forced through zero to create a simple conversion factor based on the slope of the equation between the two tissue concentrations. Where necessary, data were square root-transformed to meet the assumption of residual homoscedasticity. The regressions' beta coefficients were used to create the comprehensive meta-regression; 16 regressions were derived for river otter and mink.

The models were also validated using an external data set of individual mercury tissue concentrations of animals collected from the Province of Alberta, Canada. For the validation exercise, tissues from fur, brain, kidney, liver and muscle were collected from 18 river otters and 17 mink. The samples were dissected, mechanically homogenized and freeze-dried, and mercury concentrations were measured on a direct thermal decomposition mercury analyzer (MA3000, Nippon Instrument, Texas). The validation was completed using a root mean square error (RMSE), which compares the difference between the model predicted tissue concentration and the actual concentration of that tissue. The RMSE was normalized by the range of the concentrations of Y (NRMSE). The lower the NRMSE, the more accurate a predictive model is.

Results and Discussion

All predictive models met model assumptions. The beta coefficients for all models were statistically significant (<<0.001). The models could explain between 80% and 98% of variance. Overall, the model metrics suggest the generated models have good predictive capabilities. This was confirmed by the model validation using the NRMSE. For river otter, the NRMSE was between 13% and 28%, while it was between 14% and 31% for mink. Higher NRMSE values were produced by models that used fur as a predictor, and lower NRMSEs were from models predicting one internal concentration from another. The ratios between fur and brain mercury concentrations were 0.165 for river otter and 0.177 for mink.

The current guideline levels for furbearing mammals used by the U.S. Environmental Protection Agency (USEPA) is 30 $\mu g \cdot g^{-1}$ and more conservatively 20 $\mu g \cdot g^{-1}$. Using this newly developed model, we predict the internal doses of mercury in the brain will be 4.9 $\mu g \cdot g^{-1}$ and 3.3 $\mu g \cdot g^{-1}$ dry weight for river otter and 5.3 $\mu g \cdot g^{-1}$ and 2.5 $\mu g \cdot g^{-1}$ dry weight for mink. These levels are within the lowest observable effects level of 3-5 $\mu g \cdot g^{-1}$ dry weight that was reported to alter brain neurochemistry (Dornbos et al. 2013). Therefore, a more conservative guideline may be needed. The derived conversion factors for mercury concentrations in fur versus internal organs, based on these models, allow for the estimation of tissue residue levels using non-invasive matrices for environmental monitoring programs. For example, biomarker samples can be collected non-invasively from hair snags, road kill, or pelts from trappers at auction. The purpose of these models is to provide a screening tool to assess the mercury levels in an aquatic environment. These biomonitoring data can be used by environmental and public health professionals to develop management programs.

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Health of leopard frogs in Hamilton Harbour: A caging study (PL)

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Cootes Paradise is a coastal wetland, located at the west end of the Hamilton Harbour Area of Concern in western Lake Ontario, that has been heavily impacted by population growth, industrialization and land use changes that have impaired water quality. Stressors include excess nutrients from agricultural runoff and inputs of contaminants from an upstream wastewater treatment plant and combined sewer overflows from tributaries draining into the marsh. Since municipal effluent can contain hormones and pharmaceuticals and personal care products (PPCPs) that may act as endocrine-disrupting chemicals, exposure to these compounds may result in adverse effects for aquatic wildlife. To examine this more closely, an *in situ* caging study was conducted at multiple locations in Cootes Paradise in 2014 and 2015 using northern leopard frogs (*Rana pipiens*). Frog eggs were placed into cages at study locations and one upstream reference site and then monitored for hatching success, tadpole deformities, survival to metamorphosis, and body size and deformities of newly-transformed froglets. Five replicate cages were set up at three Cootes Paradise locations in 2014 and five locations in 2015. Mean hatching success of leopard frog eggs ranged from 3% to 67% at Cootes Paradise locations in the two study years and was significantly lower at West Pond, Boathouse and Westdale Inlet, but not at the Upper Paradise Pond site, compared to the reference site. Mean percentages of tadpoles with deformities were not significantly different between Cootes Paradise locations and the reference site in both study years. Survival to metamorphosis in leopard frogs was relatively high in cages at Upper Paradise Pond and the reference site, and lower at the West Pond, Boathouse and Spencer Creek sites. No gross morphological deformities were found in any newly-transformed froglets at the end of this study. Gonads from male frogs were also examined for an intersex condition (i.e., the presence of testicular oocytes) in 2014. While only seven male frogs from West Pond could be assessed in total, three had oocytes and of these, two were exceptional in that they contained large aggregates of oocytes that were similar in structure to those found in female frogs. Nitrate concentrations in water at cage locations were consistently highest at West Pond for every biweekly collection and exceeded concentrations associated with lethal and sublethal effects in amphibians in the laboratory. Hormones and PPCPs were detected in relatively higher amounts in water at West Pond in 2014 and 2015 based on the results of Polar Organic Chemical Integrative Samplers deployed at cage locations. Effects found in frogs from West Pond (a shallow floodplain pond) may be related to increased exposure to chemical stressors, since the majority of its

water is from the wastewater treatment plant. Findings from this study will be used in the assessment of the status of wildlife in the Hamilton Harbour Area of Concern.

Health impact assessment and remediation of oil sands process-affected water, using embryonic zebrafish (PL)

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Large volumes of oil sands process-affected water (OSPW) are produced by surface mining of the oil sands in northern Alberta. Given the economic importance of the oil sands in Canada, it is vital to develop effective methods for risk assessment and remediation of OSPW. Previous studies have shown that OSPW is both acutely and chronically toxic and can be lethal at higher concentrations to aquatic organisms. However, no standard operating procedure (SOP) exists for evaluating the lethal and sublethal toxic effects of OSPW. Therefore, the first objective of the present study was to develop an SOP for assessing the toxicities of both the acid-extractable organic (AEO) fraction and whole OSPW in zebrafish (*Danio rerio*) embryos using the robust morphometric biomarkers. We then used the same SOP for estimating the "no observed effect" concentration (NOEC) following phosphate biostimulation of OSPW to evaluate if phosphate biostimulation is an effective remediation technique for improvements in tailings pond management. Some of the morphometric parameters investigated in this study included embryo length, eye diameter, yolk utilization, hemorrhage, swim bladder non-inflation, mechanical response to an external stimulus, pericardial and yolk sac edema, as well as malformation of the spine. Our results showed significant variations and sensitivities to both AEOs and whole OSPW before remediation depending on the zebrafish stage of development. Moreover, significant differences in OSPW toxicity were observed from distinct tailings ponds using these morphological endpoints. Our results also showed significant reductions in the frequency of abnormalities in the most sensitive endpoints previously identified following exposure to increasing concentrations of phosphate-biostimulated AEOs and whole OSPW compared to untreated OSPW. In addition, acute exposure to phosphate-biostimulated AEOs and whole OSPW was significantly less lethal to developing zebrafish relative to untreated OSPW. The present study identified and validated a number of sensitive morphological endpoints in zebrafish embryos that can be used for health impact assessment and toxicity monitoring of OSPW. Furthermore, our biomarkers are an effective means of estimating the efficacy of biological remediation to reduce toxicity of OSPW. Our findings also demonstrate that combination of microorganisms and phosphate biostimulation provides an effective passive method of OSPW remediation that should aid in the development of more acceptable and much needed sustainable methods of surface-mining oil sands management in northern Alberta.

Developing LA-ICP-MS analysis of selenium in pectoral fin rays of white sturgeon (*Acipenser transmontanus*) from San Francisco Bay as a non-lethal assessment technique (PL)

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There is increasing pressure to develop alternative methods to assess the hazards posed by contaminants to wildlife while also reducing the number of lethal sampling events using wild animals. Opportunistic sampling was conducted to collect tissues from angler-harvested resident white sturgeon (*Acipenser transmontanus*) in San Francisco Bay, with the ultimate goal of developing a non-lethal method to assess exposure to inorganic contaminants. Specifically, this population of sturgeon is thought to be at risk to accumulate elevated concentrations of selenium from their diet, which predominantly consists of overbite clams (*Potamocorbula amurensis*). Soft tissues (e.g., muscle, liver, or ovary) are typically analyzed to determine exposure, but ongoing excretion, metabolism and tissue redistribution preclude resolving any temporal information from these analyses. Time-resolved exposure can be determined using micro-chemical analysis of otoliths, but this requires lethal sampling. Pectoral fin rays also contain annual growth rings, but can be sampled non-lethally and require no special preservation for micro-chemical analysis. However, the relationship between trace elements in the bony matrix of otoliths and fin rays is not yet known. As part of a collaborative study, 27 and 22 adult white sturgeon were collected from San Francisco Bay in February 2015 and 2016, respectively. Selenium (Se), as well as zinc, strontium, mercury, lead and calcium (internal standard) were analyzed in annual growth zones of pectoral fin rays and otoliths from these fish using continuous LA-ICP-MS. Relationships between concentrations of Se in soft tissues (liver, muscle and ovary), otoliths and pectoral fin rays will be presented. Fin rays may represent a relatively simple non-lethal tissue sample to establish temporal exposure to several elements in white sturgeon from San Francisco Bay.

Species traits predict avian sensitivity to dioxin-like compounds (PO)

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Avian species exhibit up to 1000-fold differences in sensitivity to dioxins and dioxin-like compounds (DLCs). These sensitivity differences are directly related to the identity of two amino acids within the ligand-binding domain of the aryl hydrocarbon receptor 1 (AhR1 LBD). Surprisingly, amino acid expression within the AhR1 LBD of avian species does not appear to be explained by phylogenetic relatedness alone, suggesting factors other than phylogeny may better predict avian sensitivity to DLCs. We built on previous existing data sets of AhR1 LBD amino acid sequences by collating diverse information indicative of the biological and ecological traits of 89 (largely North American) bird species spanning 41 families. Boosted regression tree (BRT) analysis was used to determine which variables were most important in predicting species sensitivity (measured as the 2,3,7,8-tetrachlorodibenzo-p-dioxin half maximal effective concentration; TCDD EC_{50}). We controlled for potential phylogenetic non-independence by including phylogenetic eigenvectors as covariates. Some 74% of the variance in DLC sensitivity was explained by 67 variables, and five trait-related variables explained over half of the model deviance: duration of incubation period (22.1%), habitat associations (20.6%), duration of fledging period (9.3%), testes mass (8.1%), and migration route (6.6%). Species with a faster developmental rate,

higher sexual selection, that use terrestrial habitats and with inland migration routes or non-migratory strategies show greater sensitivity to DLCs. By comparison, phylogenetic relatedness was a weak predictor of sensitivity (e.g., phylogenetic coordinate 4: 7.9%). This study is the first to reveal that life history and ecological traits correlate well with a commonly used toxicological measurement across a broad range of avian species. This presents an important alternative method to traditional single-species toxicity testing that will improve our prediction and management of contaminant risk to birds.

The effect of municipal wastewater effluent on the stress response of fish in the Bow River, Calgary, Alberta (PO)

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Municipal wastewater effluent (MWWE) discharged to aquatic ecosystems contains nutrients and pharmaceuticals and personal care products (PPCPs) that are not completely eliminated during wastewater treatment processes. As the human population grows and ages, the occurrence of PPCPs in MWWE-impacted waterways is expected to increase. This is concerning because these compounds are biologically active at low concentrations, can generate synergistic effects when present in mixtures, and their effect(s) on non-target organisms remain poorly understood. Studies in natural systems provide a means to validate the use of biomarkers of contaminant exposure and effects, which are a useful tool to identify sublethal exposure consequences of current and emerging contaminants. Previous studies with fish have demonstrated that chronic exposure to MWWE compromises their adaptive response to a secondary acute stressor by disrupting the functioning of the hypothalamus-pituitary-interrenal axis. In this study, longnose dace (*Rhinichthys cataractae*), which are native to the Bow River, were collected upstream and downstream of two wastewater treatment plants. These small-bodied fish are excellent sentinels because they are abundant, have small home ranges, and therefore reflect the state of their local environment. The results highlight the use of molecular and phenotypic endpoints related to the cortisol stress response to investigate possible stress-related impacts of MWWE on fish in the Bow River. Acquiring baseline knowledge of the impact of MWWE on native fish species will help identify current challenges with safe wastewater discharge, validate new biomarkers of contaminant exposure, and help maintain healthy ecosystems and biodiversity of our waterways. This study was supported by the Natural Sciences and Engineering Research Council of Canada Strategic Grant (to Vijayan), and by the Alberta Conservation Association through the ACA Grant in Biodiversity (to Lazaro-Côté).

Railway-associated attractants as potential toxicants for wildlife (PO)

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Many studies have investigated contaminant levels in roadside soils and vegetation for their potential effects on wildlife populations, but few similar investigations have occurred for railways. This is surprising because railway environments are known to contain pollutants and are also known to attract wildlife. This attraction stems from spillage of agricultural products, which leak from hopper cars, as well as from palatable vegetation, such as dandelions and other photophilic plant species that thrive in disturbed areas. Both sources of food can provide important supplements to wildlife populations in the vicinity of railways; however, these rail-associated foods potentially contain high levels of pollutants.

Rail-associated pollutants are relevant to wildlife conservation, especially when they occur in protected areas and involve threatened populations. Both contexts apply to grizzly bears (*Ursus arctos*) in Banff National Park, which are known to forage extensively along the rail and to consume both spilled grain and rail-side vegetation. Although studies have addressed the attraction to rail-based foods as a potential contributor to bear-train collisions, the consequences of consuming the forage itself have been largely overlooked. We examined three kinds of pollutants that potentially occur in rail-side plants and/or train-spilled grain: heavy metals, polycyclic aromatic hydrocarbons (PAHs), and mycotoxins. We collected grain and dandelion samples at four locations along the Canadian Pacific Railway in Banff National Park and grizzly bear hair from 10 individuals that were captured for another study. Our preliminary results reveal large effect sizes for grain and dandelion contaminant samples when compared to controls, and that train-spilled grain harbours comparatively elevated levels of contaminants. For example, the average sum of 16 PAHs in train-spilled grain was over 800 times the average sum for dandelions. Conversely, mycotoxin samples were largely below detection limit. Analyses of bear hair demonstrated large variation in metal concentrations between individuals, with males having overall higher levels of exposure than female bears. Our results suggest that rail-based food resources may pose a health risk for bears and other wildlife that forage along railways.

Effects of salinity on mercury in fish and invertebrates in the Saint John River estuary (PO)

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Although methylmercury (MeHg) can be higher in freshwater than in estuarine or marine sediments, it is not known whether MeHg in biota also follows this trend. The objective of this research was to determine if MeHg concentrations in fish and invertebrates decrease along increasing salinity gradients in estuaries, and whether salinity can be used as a predictor of MeHg in these animals. Ten fourspine stickleback (*Apeltes quadracus*) as well as snails and amphipods were collected from each of ten sites along the Saint John River estuary from August to October 2015. Total mercury (THg; used as a proxy for MeHg) was measured in whole stickleback and MeHg was measured in pooled invertebrates. Their stable sulfur (δ34S), carbon (δ13C) (measures of food sources) and nitrogen isotope values (δ15N) (measure of trophic position) were also determined, as these help predict variability in MeHg and THg in animals. Generalized linear models were used to determine which factors (salinity, dissolved oxygen (DO) and mean length and stable isotope values) predict THg in stickleback and which factors (salinity, DO and stable isotope values) predict MeHg in snails and amphipods. Salinity and DO of the sites ranged from 0.1 parts per thousand (ppt) to 11.1 ppt and from 737 to 1221 $mg \cdot L^{-1}$, respectively. Concentrations of THg in stickleback were 0.02 to 0.92 $\mu g \cdot g^{-1}$ wet weight across sites, and males had significantly lower THg than females ($p<0.001$). MeHg ranged from 0.03 to 0.11 $\mu g \cdot g^{-1}$ wet weight in amphipods and from 0.03 to 0.09 $\mu g \cdot g^{-1}$ wet weight in snails across sites. For stickleback, mean THg was best predicted by mean length, δ15N, DO and δ13C. MeHg in both amphipods and snails was significantly predicted by δ15N. Although several factors affect the accumulation of MeHg in the tissues of fish and macroinvertebrates, these preliminary results do not suggest salinity was a main influence. Additional field work at these sites is planned for 2016.

Assessing Natural Variability: Dare to be (Quantifiably) Different

Successes and challenges encountered in defining natural variability for water quality in northern aquatic systems (PL)

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Characterizing natural variability in aquatic systems is necessary for differentiating anthropogenic and natural changes. Regulatory boards in the Northwest Territories are clear that monitoring programs are to be developed such that they provide a solid foundation for detecting change, assessing impact, and implementing adaptive management. The benefits of adequately characterizing natural variability are clear and widely accepted among stakeholders; however, the approach to defining this is widely variable. Some variation in approach may be warranted, depending on data type and availability. The presentation will outline successes and challenges encountered in defining natural variability within the northern regulatory framework. Topics will be related to data collection, filling data gaps, consistency in application and flexibility in methodology, followed by recommendations for ongoing collaboration on this topic. Participants will be encouraged to provide input and perspective from their work in furtherance of this topic.

Testing against "normal" with environmental data (PL)

Bruce Kilgour[1], *Keith Somers*[2], *Kelly Munkittrick*[3], *Tim Barrett*[4] *and Anthony Francis*[1]

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A normal range is some fraction of reference data deemed to represent an expected condition. Limits on normal ranges are estimated with error, like other statistics (e.g., mean), which varies depending largely on sample size. Direct comparison of a sample to estimated limits of a normal range will therefore, with some frequency, lead to incorrect conclusions about whether a sample is inside or outside the normal range when the sample is near the limit. Those errors can have significant costs and risk implications. When reference data are normally distributed, bounds on normal ranges can be computed using non-central distributions of test statistics (i.e., t-test). In this paper, we illustrate the calculation of tolerance limits that test whether samples are within or outside the normal range using benthic data from the Steepbank River.

Definition of normal range using box plot analysis (PL)

David Huebert[1] *and Mike Sanborn*[1]

[1]*AECOM*

Understanding the current baseline chemistry of water and sediment is critical for developing informed environmental decisions. Accurate description of the aquatic environment is an essential component of environmental impact assessment for project development and, later, for establishing operating licence requirements. During operations, definition of normal range allows for recognition of any downstream effects for projects with effluent discharge. Determination of current conditions is also

required during site remediation, where decisions regarding the level and extent of mitigation are dependent on delineation of contamination. However, developing an understanding of normal range is a challenging undertaking for a number of reasons. Environmental chemistry data are often not normally distributed, which means that data transformations are required prior to use of parametric statistics. Analytical detection limits often result in reporting of non-detect data, which are difficult to incorporate into analytical processes. This problem is compounded when detection limits change over time. Sets of data often contain outliers, which may or may not be due to measurement error, and which can profoundly affect mean and standard deviation. Finally, water and sediment chemistry can change over time and space, either seasonally or over a period of years. One approach to definition of normal range that is capable of dealing with these analytical challenges is the use of box plots for data analysis. Box plots are non-parametric and so do not require normally distributed data. They can also accommodate a considerable amount of non-detect data, and provide a graphical understanding of analytical limits related to these data. Finally, box plots provide a consistent definition of outliers that does not depend on data distribution, but rather on the characteristics of the data themselves.

Predicting future observations by applying iterated bootstrapping to existing data sets (PL)

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A central role of environmental monitoring is identifying meaningful change. This task can be complicated in many ways, but can often be simplified into separating expected, or probable, differences from those that would be unexpected, or improbable, if the conditions of the study system are stable. Many techniques are used to examine this question. Many rely on inferential statistics and p-values to accomplish this, while others also include an estimated Critical Effect Size (CES). Environmental Effects Monitoring (EEM) is a good example of an approach that uses both. Although the CESs used in EEM are generally applicable and are an important step, they may be uncertain in specific scenarios. Unfortunately, we cannot specifically identify when CESs are useful and where they may not be. There are, however, some characteristics, such as small sample sizes, that can clearly affect their validity. While several remedies are available to address uncertainty, including the integration of confirmation cycles into monitoring, further refining CESs can also contribute to error reduction. Iterated bootstrapping is one option that offers many advantages over other trigger development techniques. Intervals calculated from single bootstrapped intervals often have high coverage accuracy and beneficial convergence properties, but not always. Both aspects do, however, improve with double or triple bootstrapping. Iterated bootstrapping can, however, be computationally heavy and requires estimating resample (and re-resample) sizes. While not infallible, iterated bootstrapping may be a powerful tool in an adaptive monitoring program.

A complicated life in Reversing Falls: The dynamic tale of Environmental Effects Monitoring and Irving Pulp and Paper, Ltd. in Saint John, New Brunswick (PL)

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Irving Pulp and Paper, Ltd. (IPP) discharges mill effluent into the Reversing Falls, a unique hydrological feature located at the confluence of the Saint John River and the Bay of Fundy. The

magnitude of the Bay of Fundy tides forces the falls to reverse its flow direction twice each day, resulting in an extremely dynamic estuarine environment with wide swings in flow direction and velocity, water depth, temperature, and salinity. A two-dimensional plume delineation, conducted prior to the initial Environmental Effects Monitoring (EEM) program, suggested that the 1% effluent zone for IPP extended several kilometres upriver on the rising tide and also downstream on the falling tide into Saint John Harbour. IPP is located in an industrial urban setting, in which there are other historical and current municipal and industrial wastewater discharges that may also influence the aquatic environment. These factors add to the complexity of EEM study designs and data interpretation. This presentation will trace the path of IPP through the federal EEM decision framework for fish and the benthic invertebrate community (BIC) to its current status and looking forward. For the fish component, IPP recently completed a field study and pilot mesocosm studies, and then shifted to Investigation of Cause (IOC) and Investigation of Solutions (IOS). IOC work conducted during the recent (2013-16) EEM program re-evaluated historical field data when BIC effects were determined. Non-mill effluent sources in the vicinity of IPP at the time of the historic field studies included a number of untreated municipal effluent sources, which have recently been consolidated, treated, and shut off as part of a major harbour clean-up effort. Additional non-mill sources are identified and described in relation to their potential to influence the BIC. The recent EEM program identified the highly dynamic natural environment as perhaps the major force shaping the BIC structure near Reversing Falls. A better understanding of the vertical and horizontal dispersion of the IPP effluent plume using three-dimensional modelling would be helpful in advancing IOC for the BIC towards potential solutions for fish and the BIC.

Adaptive management in Canada's Northwest Territories: A case study of cesium and thallium in fish tissue at a diamond mine (PL)

Rainie Sharpe[1], Melanie Jaeger[1], Clayton James[1], Hilary Machtans[1], Peter Chapman[2] and Alexandra Hood[3]

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Recent guidance from the Northwest Territories (NT) Water Boards has encouraged incorporation of an adaptive management approach towards aquatic effects monitoring. The successful implementation of adaptive management requires the inclusion of biologically and ecologically meaningful endpoints to serve as action-level triggers, and an understanding of the magnitudes of change in these endpoints that are indicative of natural variability versus change that is due to anthropogenic influence. A temporal-reference-normal range decision matrix was first implemented at the Snap Lake diamond mine as part of the fish tissue chemistry component of the mine's Aquatic Effects Monitoring Plan (AEMP) in 2013. Low action levels for cesium and thallium were triggered in lake trout (*Salvelinus namaycush*) and round whitefish (*Prosopium cylindraceum*) in 2013, and in lake chub (*Couesius plumbeus*) in 2015 for the same metals. The incorporation of normal range, a quantified estimate of natural variability, into the decision matrix strengthened the confidence with which the action levels were triggered, and successfully identified the need for further investigation into the cause of an increase in thallium and cesium concentrations in fish tissue at Snap Lake. The results of the investigation following the cesium and thallium low action-level trigger will be discussed, and the concept of normal range defined as a prediction interval will be presented.

Analytical Developments

Measurement of dissolved trace-metal species distributions at environmentally relevant concentrations under *in situ* conditions using field-flow fractionation coupled to ICP-MS (PL)

Chad Cuss[1], Iain Grant-Weaver[1], Mark Donner[1], Muhammad (Babar) Javed[1], Tommy Noernberg[1], Nilo Sinnatamby[1] and William Shotyk[1]

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In aquatic systems, the toxicity of a substance is limited by its bioavailability. Trace metals are distributed among the particulate (>0.45 µm) and dissolved phases (<0.45 µm), where dissolved trace metals are more bioavailable for most aquatic organisms. Within the dissolved phase, the "truly dissolved"/primarily ionic species <1 nm (≈1 kDa) in size are the most bioavailable. However, measuring the relative contribution of different dissolved metal species at environmentally relevant concentrations has been challenging due to inadequate limits of detection (e.g., ion-selective electrodes) or problems with interferences in complex natural matrices (e.g., voltammetry). These limitations have led to a reliance on unverified computational models for estimating the concentrations of free species and the development of regulations based on total or dissolved metal concentrations, which are not always adequate indicators of toxicity. Recently, these challenges have been overcome by the combination of advances in analytical methods for size separation at the nanometre scale (i.e., asymmetrical flow field-flow fractionation; AF4), coupling to instruments designed to measure metals at low concentrations (i.e., inductively-coupled plasma mass spectrometry (ICPMS)), and specialized methods and conditions for sampling and analysis (i.e., the ultra-clean, metal-free SWAMP lab facility at the University of Alberta). Importantly, the tunable carrier fluid used in AF4 enables the control of key variables such as pH and ionic strength, so that the natural distribution of dissolved metal species is preserved during analysis. This presentation will discuss the necessity of clean sampling techniques/facilities and the development of a method for determining the distribution of dissolved metal species at environmentally relevant concentrations using AF4-ICPMS. The value of using actual measurements of dissolved metal species to estimate bioavailability/toxicity will be highlighted. Preliminary results from the analysis of samples collected from the lower Athabasca River and its tributaries near bitumen mining and refining operations will also be presented.

Chemical speciation of arsenic and selenium in aquatic systems: Analytical techniques and their application (PL)

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Arsenic (As) and selenium (Se) are potentially toxic elements that are of concern in aquatic systems due to their known toxicological effects and potential to be mobilized in high concentrations by natural or anthropogenic influences. The unique chemistry of both elements sets them apart from other "heavy metals" as their environmental fate and behaviour, including toxicity, is largely determined by their chemical speciation. While measurements of total As and Se provide important preliminary information, chemical speciation is a crucial component of any study assessing the toxicity of these elements. These

measurements are, however, afflicted with unique analytical challenges that become critical when working at trace (i.e., <1 $\mu g \cdot L^{-1}$) concentrations. For that reason, many environmental monitoring agencies are unable to reliably measure these elements unless the water is severely impacted. This talk reviews some of the current analytical techniques for measuring As and Se, including their biologically relevant species. The focus will be on using modern techniques such as ion chromatography paired with inductively coupled plasma mass spectrometry equipped with dynamic reaction cell (IC-ICP-DRC-MS). Recent results from a study of water quality in the lower Athabasca River will be used to demonstrate the importance of As and Se redox state speciation and how it relates to other water quality parameters. Analyses from other aquatic systems (natural and industrial impacted) will also be summarized and used as examples.

Trace metal distribution in dissolved and particulate forms in the lower Athabasca River (PL)

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In recent years, there has been an increasing ecological and human health concern associated with aquatic trace metals. Understanding of trace metals bioavailability in water is important to predict metals toxicity to aquatic life, because metals can be partitioned into dissolved (<0.45 µm) and particulate (>0.45 µm) forms, where dissolved forms are considered more bioavailable. It has been claimed that open pit mines and upgraders of the Athabasca Bituminous Sands in Alberta, Canada, are a significant source of silver (Ag), cadmium (Cd), lead (Pb), antimony (Sb) and thallium (TI) to the Athabasca River (AR). As a part of a big project to resolve natural versus anthropogenic inputs to the lower AR, this study was initiated. In 2014, AR surface water samples were collected from 13 sites along the main stem of the AR, beginning upstream from Fort McMurray, within the industrial zone, and travelling downstream toward the Firebag River. In 2015, water samples were collected from all the 13 sites sampled previously plus from some new sites. The main objective of this study was to determine overall trace metal concentrations in the AR water, any potential impact of industrial activities, and metal distribution in dissolved and particulate forms. Further fractionation of particulate trace metals into different chemical forms was performed to predict any potential release of trace metals into AR water. The results showed that total (dissolved+particulate) concentration of most of the potentially toxic metals (Cd, Pb, TI) as well as the trace metals known to be enriched in bitumen (vanadium (V), nickel (Ni), molybdenum (Mo), rhenium (Re)) in the AR water were lower than the Canadian Council of Ministers of the Environment's (CCME's) Canadian Water Quality Guidelines for the Protection of Aquatic Life. The dissolved concentrations of Cd, Pb and TI were also low compared to the U.S. Environmental Protection Agency (USEPA) guidelines for the protection of aquatic life. None of the total and dissolved concentrations of any of the trace metals showed any significant difference between upstream, downstream, and within the industrialized zone. Comparing dissolved versus total metals in the AR water over the year, the dissolved metal concentrations remained unchanged. For example, in 2014, dissolved Pb was ~23 $ng \cdot L^{-1}$, which was similar to the average dissolved Pb (~26 $ng \cdot L^{-1}$) in 2015. However, total metal concentrations varied significantly: ~300 $ng \cdot L^{-1}$ Pb was found in 2014 compared to ~60 $ng \cdot L^{-1}$ Pb in 2015. This difference in total concentrations is mainly because of the abundance of suspended solids in the water. Comparing dissolved versus particulate metals, ~80% of most of the total metals (Ag, arsenic (As), beryllium (Be), Cd, chromium (Cr), Pb, TI) were found in particulate form. The metals fractionation showed that insignificant concentrations of trace metals were present in easily available forms, whereas a huge proportion of metals were found in mineral bound forms which are stable under natural environmental conditions. These results provide overall trace metals status in the lower AR water and

have implications for understanding the trace metals mobility and bioavailability in river waters around the world.

Application of ultraclean lab methods for the determination of dissolved trace metals in the lower Athabasca River: Challenges, opportunities and new perspectives (PL)

William Shotyk[1] *and Beatriz Bicalho*[1]

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It is well known that typical concentrations of many trace metals of environmental significance (e.g., silver (Ag), cadmium (Cd), lead (Pb), antimony (Sb), thallium (Tl)) in ancient polar ice, open ocean and deep seawater, as well as uncontaminated groundwaters, are extremely low: at the levels of $ng \cdot L^{-1}$ and below. Measuring trace metals reliably at these levels requires not only tremendous analytical sensitivity, but more importantly, "ultraclean" lab procedures and protocols to minimize the risks of contamination from ambient air, reagents, containers and any surfaces (syringes, filters, tubing) that come into contact with standards and samples. Avoiding metals and metal alloys, as well as glass, are obvious important steps in the process, but not all plastics are created equal: some leach more metal than others (e.g., vanadium from high-density polyethylene (HDPE)), and none is perfect. All materials coming into contact with the sample must be acid-cleaned, and establishing clearly defined blank values and limits of detection is a crucial part of the analytical process. In this presentation, we provide examples of these procedures and protocols from the new, ultraclean, metal-free SWAMP lab at the University of Alberta for the study of trace metals in soil, water, air, manures, and plant: the design of the lab itself and the strategy for maintaining clean air, the use of metal-free, laminar flow Class 100 clean air cabinets for additional air filtration, the production of sub-boiled nitric acid by distillation in high purity quartz for cleaning of plastic-ware and acidification of water samples, and the choice of filters, syringes and sampling containers to minimize leaching of trace metals. We further show how we have applied these techniques to better understand trace metals in the surface and groundwaters of the lower Athabasca River where there are growing concerns about environmental impacts from upgrading and refining of bitumen. In fact, in the mainstem of the river, both upstream and downstream from industry, trace metals such as Ag, Cd, Pb, Sb and Tl are all found at very low levels: at or below the levels reported for bottled waters. For example, in some tributary streams, Pb may be present at the same level ($5\ ng \cdot L^{-1}$) as ice from the Canadian Arctic more than 5000 years old. The results obtained to date using ultraclean lab methods suggest that previous work on metals in the environment of the lower Athabasca watershed have overestimated human impacts by a considerable margin.

Enhanced analytical techniques for keeping track of crude oil in water environments (PL)

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When crude oils are spilled in water environments, hydrocarbon bioavailability both immediately after a spill and over the long term is of concern. It is important to know benzene, toluene, ethyl benzene, and xylenes (BTEX) and polycyclic aromatic hydrocarbon (PAH) contents of the crude oils. However, to understand toxicity, it is also important to understand how evaporation, dissolution, biodegradation and oil dispersion impact actual hydrocarbon contents in the water phase, both in the immediate and long term. Analytical techniques for hydrocarbons have focused on quantifying saturate

and aromatic species up to 40 carbons (C40) in size. However, crude oils consist of hydrocarbons up to and larger than C120. Keeping track (closing mass balance) of crude oil hydrocarbons as they evaporate, biodegrade and interact with sediment is necessary to understand the timing and relative rates of these processes that move hydrocarbons into and out of the water system. The timing of these processes after the spill event will likely have different toxic impacts for different biological systems. Analytical methods will be discussed and results shown for tank-scale tests of diluted bitumen products and conventional crude spilled into fresh water containing sediment.

The importance of untargeted analysis for the environmental monitoring program in the Alberta oil sands region (PO)

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In northern Alberta, Canada, the production of bitumen from open-pit oil sands mining requires 2-3 barrels of water for every barrel of bitumen produced. Large volumes of oil sands process-affected water (OSPW) are stored in large tailings ponds so that it can be recycled into the extraction process. Despite the water recycling efforts (80-95%), in 2012 the use of fresh water was approximately 187 million m^3 (Canadian Association of Petroleum Producers 2013), which is about 40% of the City of Toronto's annual water consumption. Moreover, due to the large water volume used daily, tailings ponds are growing in volume and number and, in 2011, covered approximately 170 km^2. There are concerns that OSPW leaches from tailings ponds into groundwater, or into river water, but proving this is complicated by the fact that natural groundwater can contain many of the same chemicals as OSPW (e.g., naphthenic acids). As a consequence, there is an ongoing need to improve environmental monitoring in the Athabasca region. Due to the complex nature of OSPW, an untargeted analytical platform to interrogate and interpret the large volume of data generated during the analysis of water samples by LC-Orbitrap-MS (operated with resolution power from 120K to 480K) was developed. The approach was applied to river water samples collected in the Alberta oil sands region. Results indicate that the approach extends monitoring capabilities from the “classical” naphthenic acids to the inclusion of ionisable compounds with molecular formula CcHhNnOoSs. It has recently been postulated that compounds belonging to this general class contribute to the toxicity of OSPW. Thus, obtaining information about these compounds will enable regulators and environmental managers to improve monitoring and reclamation programs in the region.

Analysis of ethoxylated surfactants and phosphates/phosphite in hydraulic fracturing flowback and produced water by using LC-Orbitrap-MS/MS (PO)

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Hydraulic fracturing (HF) has emerged as a revolutionary method of unconventional oil and gas recovery. The toxicity of HF flowback and produced water (FPW) has not been previously reported and is complicated by the combined complexity of organic constituents in HF fluids and deep formation water. Untargeted HPLC-Orbitrap-MS/MS revealed numerous unknown dissolved polar organics in an FPW sample from Alberta, Canada. Two series of ethylene oxide (EO) surfactants, polyethylene glycols (PEGs, $H(OC_2H_4)_nOH$) and unsaturated polyethylene glycols (two double bonds) ethoxylates (uPEGs), were

identified in hydraulic fracturing flowback and produced water. The PEG surfactants are polymers of EO units, and those identified here included PEG-EO4 to PEG-EO13 (i.e., n=4-13), similar to the results of Thurman et al. (2014). Here, all the PEG-EO*n* species were not only confirmed by accurate masses of the protonated or ammonium adducted ions, but moreover by diagnostic fragmentation in MS/MS experiments. However, PEGs were not the only important features in the chromatogram of the current sample. Four phosphates and one phosphite were identified based on accurate mass (mass accuracy <2 ppm) and putative molecular formula. The further structural elucidation was proofed by MS/MS experiments and confirmed by using the authentic standards. For example, there was consecutive loss of C_4H_8 of tris(2,4-di-tert-butylphenyl)phosphate at low collision energy and further loss of phenyl groups from a phosphate core at higher collision energy. This allowed a general structure to be confidently proposed as an alkyl substituted tri-dibutylphenyl phosphate ester, with later confirmation with an authentic standard. This phosphate chemical is not listed on Canada's Domestic Substance List (DSL), however, a corresponding tris(2,4-di-tert-butylphenyl)phosphite ($C_{42}H_{63}PO_3$) is a common polymer antioxidant on the DSL and would yield the detected phosphate upon oxidation. These phosphates are known flame-retardants used as polymer additives but also in fire-resistant hydraulic fluids and in lubricants. Alkyl phosphate esters were previously reported in FPW samples, but no aryl phosphate esters have been previously reported to our knowledge. This technique is applied for profiling analysis of flowback and produced water samples to monitor water quality that results from fluids used in hydraulic fracturing.

Effective Communication in Ecotoxicology

Effective communication in ecotoxicology (PL)

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The closing session of last year's Canadian Ecotoxicity Workshop (CEW) in Saskatoon featured a new, interesting and challenging student competition called the "Three Minute Thesis" (3MT®). This 3MT® competition is now being considered for future CEWs. The challenge in this competition was/is for participants to present their work as effectively, concisely and interestingly as possible—in a simple format—to a diverse, but educated, audience. Even those who have made many presentations at this and other scientific conferences for years were impressed with the quality, format and wide appeal of these presentations: good science delivered in simple, interesting, and in some cases, novel ways. In contrasting these presentations to the traditional format of communicating our work, a higher-level question emerging from this was: "Are we doing the best job we can at communicating the science of ecotoxicology?" This presentation will provide an historical perspective on how we traditionally communicate our science to our colleagues, our peers, and to the public. The presentation will explore various improvements to the effective communication of content in both platform and poster formats, panel discussions, and interactive sessions, with the objective of making our science more accessible to stakeholders. Moreover, the presentation will highlight the use of new and emerging tools and technologies such as interactive presentations (including videos), webinars, presentation software, and poster formatting.

Effective presentations: A business development perspective (PL)

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"If you can't explain it simply, you don't understand it well enough." These simple words from Albert Einstein can serve as a template for effective presentations. In general, scientific presentations focus primarily on data and results and lack emphasis on the bigger picture. These presentations commonly provide narrow glimpses of the project instead of the entire story or context, resulting in a failure to resonate with the broader audience. The information transcribed from the laboratory to business development to the end user changes dramatically with the change in audience. The information the end user needs or values differs from how it is presented at the bench level. At the core, presentations should be a storytelling initiative and an opportunity to discuss the bigger picture. Through past experience with marketing, account management and business development roles, I have worked closely with laboratory personnel to craft value propositions for clients and businesses. The goal of this session is to provide lessons and takeaways to enhance presentation skills within the scientific community. More importantly, the hope is that presenters become storytellers, such that the non-scientific community understands the importance of the data being presented and the information is shared in way that is engaging and hits home to each individual person. In short, what is your elevator pitch for your research?

When only zero risk is acceptable: Bridging the gap between public and scientific understanding of risk (PL)

Stella Swanson[1]

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Scientists and the public can have very different perspectives about risk, especially the level of risk that is considered acceptable. Scientists are often surprised and frustrated when their carefully constructed risk assessment results are regarded as untrustworthy, irrelevant, or just plain wrong. The public are often adamant that no risk is acceptable. This is especially true for risks associated with high uncertainty, high dread, and/or a perceived low level of public control (e.g., nuclear projects). It is also true for situations where risks are distributed over large temporal and spatial scales, persistent, or delayed. Discrepancies between those who enjoy the benefits and those who bear the risks are another key factor in risk acceptability. However, the most fundamental cause of low or zero risk tolerance is the violation of social or cultural interests and values. Assessment and management of complex risks in a high-value environment requires discourse about risk acceptability. The determination of acceptable risk requires meaningful engagement with Indigenous peoples and public stakeholders. These discussions must acknowledge that there are different legitimate viewpoints regarding risk acceptability. There are never any guarantees; however, a risk assessment process that has involvement and input from Indigenous peoples and the public from the beginning of the process through to the characterization of the risk has a much better chance of acceptance. This engagement should start with an examination of values. Acknowledgement of values (and the bias associated with values) is fundamental to transparent decision-making. In some circumstances, values are so high and so intensely felt that any risk will remain unacceptable. It is important to recognize these situations and move on to alternatives, preferably earlier rather than later. If sufficient trust has been established via engagement in the risk assessment process, risk mitigation and the achievement of an acceptable level of risk may be possible even in high-value and high-uncertainty situations. However, benefits must be tangible, some involvement in the control of risk must be possible, and there must be credible adaptive management plans for risks that may be delayed or more widely dispersed than expected. Cumulative risks must also be considered in a meaningful manner, with no reliance on “creeping baselines”. In summary, the definition of acceptable risk always involves risk balancing and trade-offs and some risks may be deemed to be intolerable, no matter how low they are deemed to be by scientists. This is a difficult lesson to learn and accept.

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