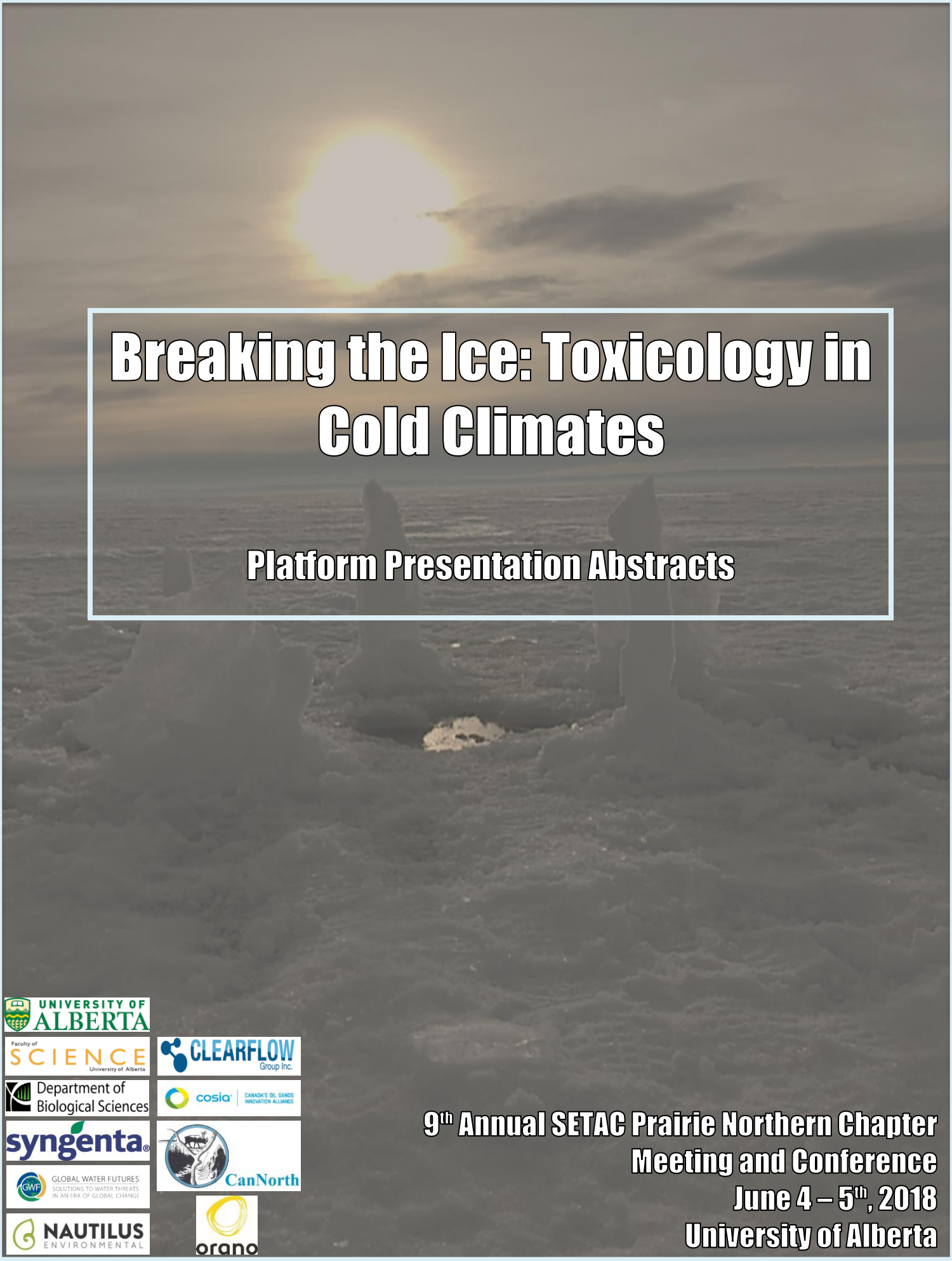

Breaking the Ice: Toxicology in Cold Climates

Platform Presentation Abstracts

9th Annual SETAC Prairie Northern Chapter Meeting and Conference
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9th Annual Chapter Meeting of SETAC Prairie-Northern

"Breaking the Ice: Toxicology in Cold Climates"

Platform Presentation Abstracts – In Order of Occurrence

1. Establishing thresholds for the effects of inorganic contaminants in northern Canadian ecosystems

Plenary: Dr. Jim McGeer
Wilfrid Laurier University

Canada's North holds abundant resources and in the next decade, exploration and development are expected to significantly increase, with a promise of economic benefits for communities. These northern environments are also pristine, considered fragile and experiencing unprecedented climate change. Therefore, a key aspect of ensuring sustainable resource development is an understanding of the potential for impacts, and establishing appropriate northern specific thresholds for environmental protection. There are important gaps in our understanding of the risks associated with contaminant exposure in northern environments. One of the most significant of these uncertainties centres on the degree to which effect thresholds for aquatic biota that have been derived for temperate (i.e. southern Canadian) environments are valid in the North. For example, animals in cold environments have lower metabolic rates, slower growth and the rates of contaminant uptake can be reduced compared to their southern counterparts. As a result, toxicity may take longer to develop and in standardized fixed duration toxicity tests (e.g. 48 h or 96 h depending on the species) the full effect of the contaminant might not be observed. Alternatively the effect of reduced temperature may be to impinge on detoxification and repair capacities. Another poorly understood feature of northern environments is the influence of geochemical conditions on effect thresholds. In this presentation we will discuss the scientific knowledge needed to better assess the risks to northern biota from contaminant exposure.

2. Potential criteria for the protection of aquatic life for water hardness using the percent change in water hardness and Ca:Mg ratio from background

Bogart, Sarah J.[1], Stock, Eric[1], Manek, Aditya[2], Tillmanns, Angeline[3], Meays, Cindy[3], and Pyle, Greg G.[1]
University of Lethbridge

An abundance of literature has shown that an increase in salinity, or the total ionic content of water, can be toxic to freshwater aquatic life, and that anthropogenic

salinization of freshwaters is increasing on a global scale. More recent research has shown that shifts in the balance of major ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, HCO_3^-, SO_4^{2-}, Cl^-) in water can also be toxic, meaning salinity alone does not fully describe risk to aquatic life. Despite this, and although a Ca water quality guideline (WQG) exists for the protection of agricultural water uses, only WQGs for Cl^- and SO_4^{2-} exist for the protection of aquatic life. Still, increases in the Ca^{2+} and Mg^{2+} content of water alone (i.e. water hardness) can be toxic to aquatic organisms. Moreover, Mg has been shown to be more toxic than SO_4. Anthropogenic sources that have the potential to increase water hardness in receiving waters to potentially toxic concentrations include, e.g., coal mining effluent and produced waters from oil and gas extraction. This presentation presents the challenges associated with and a method for the derivation of potential criteria for water hardness using percent change from background condition in both water hardness and in the Ca:Mg ratio.

3. The interactive effect of sediment bound copper and an artificially created forest fire runoff on *Hyalella azteca*

Raegan D. Plomp, Jaimie L. Klemish, and Greg G. Pyle
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Metal contamination of freshwater environments is a growing concern. Freshwater environments can become contaminated by metals through industrial operations, urban runoff, and from wastewater treatment plant effluent. At nutritive concentrations, copper is essential to aquatic organisms. However, at higher concentrations, copper is well known to be a powerful toxicant. Recent work in our laboratory has demonstrated that copper toxicity is potentiated in the presence of certain hydrocarbon contaminants, such as polycyclic aromatic hydrocarbons (PAHs) phenanthrene and phenanthrene quinone. These can occur in surface water runoff after wildfires. Because surface water runoff from burnt areas may contain high levels of organic carbon, which can sequester free metal ions, and pyrogenic PAHs, this runoff can either ameliorate or exacerbate copper toxicity to freshwater biota. This study examines the single and combined effects of pyrogenic runoff and copper to freshwater scuds, *Hyalella azteca*. Organisms of mixed age were exposed to binary mixtures of 225 mg/kg sediment bound Cu with various dilutions of an artificially created forest fire runoff (FFR) (12.5%, 25%, 50%, 75%, and 100%) for 14 days. The mixture of sediment bound copper with 12.5% FFR resulted in a more-than-additive reduction in survival. However, there was no reduction in weight for the mixture compared 225 mg/kg Cu. All other mixtures resulted in 100% mortality.

4. The osmotic effect of the hyper-saline hydraulic fracturing fluid on the rainbow trout, *Oncorhynchus mykiss*.

Delompré, Perrine[1,*]; Blewett, Tamzin[1]; Glover, Chris[1,2]; and Goss, Greg[1,3].
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Hydraulic fracturing is an industrial process allowing access to unconventional resources (oil and gas) that generates a wastewater (Flowback and Produced Water; FPW). FPW contains high salts (8X seawater), metals, and organics. FPW being hyper-saline, spills in freshwater environments have the potential to induce grave repercussions on the aquatic ecosystem. In a recent study, morphological changes have been observed in the rainbow trout *Oncorhynchus mykiss* after a 48h-acute exposure to 7.5% FPW, indicating an osmotic stress. However, the long-term effect of FPW exposure is still unknown. The aim of this research is to examine the specific salinity impact of this fluid on *O. mykiss* following a chronic exposure (up to 28d) to the raw FPW sample or its parallel saltwater-matched control solution. We examined Na^+-K^+ ATPase and H-type ATPase activities, two key enzymes implicated in osmoregulation, and measured changes in plasma ions (Na^+, K^+, Cl^-, Ca^{2+}, Mg^{2+}). Simultaneously, we paired these endpoints with morphological examination and performed gill histological analysis. This is the first study to investigate the chronic effects of FPW on *O. mykiss.*

5. An ecological risk assessment for thiamethoxam in Canadian surface waters: A weight-of-evidence approach

Mark Hanson
University of Manitoba

The neonicotinoid insecticide thiamethoxam is widely used in agriculture across North America and is detected frequently in surface waters. As a result, questions related to the ecological risk of thiamethoxam to aquatic ecosystems need to be addressed. To move our understanding forward, we used a weight-of-evidence approach to quantify the risk posed by thiamethoxam. All available toxicity data in the open literature were scored for their strength of experimental methods and the ecological relevance (survival, growth, development, and reproduction) of the responses reported. Numerical scores were assigned for strength and relevance. The scores were then used to weigh the evidence for thiamethoxam contributing to ecologically significant responses. Our focus was on Canada, where the vast majority of measured concentrations are in the ng/L range, with µg/L observed infrequently (e.g., downstream of greenhouses). There were acute data of sufficient quality to create species sensitivity distributions (SSDs) for insects, generally seen as most sensitive, as well as less sensitive invertebrates (e.g., zooplankton). Together, the weight-of-evidence and SSDs revealed little likelihood of adverse outcomes for insects from acute exposure to thiamethoxam in terms of apical endpoints in most regions. There was effectively no risk observed for zooplankton, fish, and amphibians. Chronic exposures were less well characterized, and remain a point of significant uncertainty, but still rarely exceeded available laboratory or mesocosm no observed effect concentrations. We do know rapid removal (on the order of days) is expected for thiamethoxam barring continuous inputs. We recommend that chronic exposure in the environment be better characterized, and recognize the need for well conducted mesocosm studies to reduce uncertainty for pesticide risk assessors.

6. Effects of early life-stage exposure to tebuconazole on the reproductive physiology of adult zebrafish (*Danio rerio*)

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Tebuconazole is an agricultural fungicide used to control pathogenic fungi common to a wide variety of fruits, vegetables and grains through inhibition of the enzyme 14α-demethylase. It has been shown, however, that tebuconazole can have endocrine disrupting effects on nontarget species through inhibition of the enzyme aromatase, which catalyzes conversion of testosterone to 17β-estradiol. This inhibition alters concentrations of hormones that are critical for reproduction by adults and development of early life-stages. As tebuconazole use has increased in recent years, the objectives of this research are to 1) determine whether pulse exposures of early life-stages of the model teleost, zebrafish (*Danio rerio*), to tebuconazole affects the reproductive capacity of adults, 2) determine the effects of re-exposure to tebuconazole on reproductive capacity of adult fish exposed during the early life-stage, and 3) to determine the molecular and biochemical mechanisms of any persistent effects of exposure to tebuconazole during early life-stages. These objectives will be addressed through assays at two timepoints in the zebrafish's life: early life-stage (1-hour post-fertilization – 24-hours post-hatch) and sexual maturity (3 – 4 months). Embryos used in the early life-stage assay will be raised to sexual maturity in order to assess persistent effects. Preliminary results indicate the concentrations chosen, 10 μg L^{-1} and 1000 μg L^{-1}, have no significant embryotoxic effects as compared to controls (0 μg L^{-1}).

7. Society of Environmental Toxicology and Chemistry Update

Mary Reiley
SETAC North America and SETAC Global Initiatives

8. Environmental Monitoring in Canada's North: Thou Shalt Versus Thou Should

Plenary: Rainie Sharpe
Golder Associates

9. Sublethal effects of chronic exposure to chlorpyrifos on *Xenopus laevis* tadpoles

N. Baldwin[1], N. Hogan[1,2]
University of Saskatchewan

Chlorpyrifos (CPF) is a widely use organophosphate insecticide with extensive occurrence in aquatic ecosystems worldwide. Exposure to CPF is associated with

adverse effects in fish including developmental delays and abnormalities, oxidative stress, neurotoxicity, and immunotoxicity. There is much less known regarding the effects of CPF on amphibians, particularly in the context of chronic exposures. This is relevant given that presence of CPF has been linked to the decline of some amphibian populations. The objective of this study was to evaluate effects of chronic exposure to CPF on the model amphibian (*Xenopus laevis*). Individuals were exposed to CPF (0.5, 2, 8 ug/L, nominal) in a flow-through diluter from early life stage through metamorphosis (~ 50 d). There was no effect of chronic CPF exposure on morphometric endpoints including length, weight, relative liver weight or incidence of malformations. Individuals exposed to 2 ug/L CPF were at a more advanced developmental stage compared to the solvent control. Apical effects will be assessed through histopathology of key organs along with evaluation sex ratios. Expression of genes related to immunological, neurological and oxidative stress endpoints will follow. We anticipate that this work will provide new and valuable amphibian CPF toxicity data to compare to well-studied fish species.

10. The effect of copper nanoparticles on olfaction in rainbow trout (*Oncorhynchus mykiss*)

Razmara, Parastoo[1], Mohaddes, Effat[1], Lari, Ebrahim[1], Zhang, Yueyang[2], Goss, Greg[2], Pyle, Greg[1]
University of Lethbridge

Fish rely on olfaction for survival, growth, and reproduction. Although copper ions (Cu^{2+}) have drawn the most attention in olfactory toxicology, the impact of copper nanoparticles (CuNPs) on fish olfactory systems has not been well studied. The objective of this study was to investigate time-dependent effects of CuNPs and Cu^{2+} on olfactory acuity and olfactory–mediated behaviours of rainbow trout. To establish CuNPs or Cu^{2+} induced olfactory-impairment thresholds, inhibitory concentration curves were determined using electro-olfactography (EOG). Afterwards, fish were exposed to CuNPs or Cu^{2+} at concentrations known to impair olfaction by 50% (320 and 7 µg/L CuNPs and Cu^{2+}, respectively) for a 24 h or 96 h exposure period. The response of fish to a social cue (taurocholic acid) was studied using EOG and a choice maze behavioural assay. Results of EOG revealed that while a 96 h exposure to CuNPs caused significantly greater impairment of fish olfactory function relative to a 24 h exposure to the same concentration, fish olfactory acuity partially recovered after 96 h under continuous Cu^{2+} exposure. Behavioural responses of rainbow trout to the social cue supported the results of neurophysiological experiments. Over the same exposure periods, CuNPs caused progressive deterioration of olfactory acuity, whereas at least a partial olfactory recovery was documented for continuous Cu^{2+} exposure.

11. Oil Sands Process-Affected Water: Toxicity Attribution and Evaluating Ageing as a Remediation Strategy

Gault, Ian;[1] Sun, Angela;[1] and Martin, Jonathan;[2]

University of Alberta

Oil sands process-affected water (OSPW) is used to separate bitumen from sand in the surface-mining oil sands industry of Alberta. OSPW contains a complex dissolved organic mixture that is toxic, persistent, and largely uncharacterized. One reclamation strategy consists of ageing OSPW in end-pit lakes such that sedimentation and biodegradation of the organics will eventually allow for safe environmental integration. This investigation contributes to predicting the effectiveness of this strategy by testing the toxicity of candidate chemical classes in various fresh and aged OSPW. Chemical fractionation and orbitrap mass spectrometry were used on OSPW, with cytotoxicity measured through real-time cell analysis of HepG2 cells and endocrine disruption measured using the yeast estrogenic/androgenic screens. An isolated fraction containing naphthenic acids (NAs) was responsible for the cytotoxicity observed, while the non-acidic fraction, speculated to contain steroidal chemicals, was not cytotoxic. Estrogen/androgen receptor antagonists were in all fractions, with both NAs and the non-acidic fractions active near environmentally-relevant concentrations. Toxicity of OSPW in an end-pit lake decreased over time, but an older, geographically-separate sample of 23-years had a unique time-dependent biphasic profile. Cytotoxicity decreased with ageing, with NAs responsible, but the biphasic response from the recalcitrant organics should be explored. Furthermore, multiple chemical classes may be steroidal. By identifying the most active chemicals in OSPW there can be better monitoring to ensure releases are protective of downstream uses.

12. Acute lethality of sediment-bound diluted bitumen to freshwater amphipods (*Hyalella azteca*)

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University of Lethbridge

Bitumen that is extracted from the Alberta oil sands is transported as diluted bitumen (dilbit) via pipelines that are often in close proximity to freshwater ecosystems. When dilbit is spilled into an aquatic environment, the diluents will evaporate rapidly, thereby increasing the viscosity and density of the dilbit. The remaining weathered dilbit can combine with fine sediment in the water column and sink. Weathered sediment-bound dilbit (WSD) can affect freshwater organisms by physically coating them or by releasing water-soluble organic compounds, such as naphthenic acids and polycyclic aromatic hydrocarbons and their alkyl-derivatives. However, research to date has focused on the toxicity of the water-soluble fraction of fresh, unbound dilbit to aquatic organisms. Here, we present the first study where the toxicity of WSD is assessed in a freshwater organism.

Adult freshwater amphipods (*Hyalella azteca*) were exposed for 96 hours to substrates where 100, 50, 25, 12.5 and 6.25% of the substrate by volume was WSD. All concentrations of WSD resulted in 100% mortality. Subsequent exposures show the water-soluble fraction of WSD does not result in significant mortality and does

not affect the natural negative phototactic behaviour of amphipods. Ultimately, this research presents novel methods to expose freshwater invertebrates to WSD and is an important step towards developing spill management guidelines that can be implemented in future dilbit spills.

13. Impacts of *in vitro* exposure to oil sands process-affected water and its fractions on mammalian immune cells.

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University of Alberta

Natural Resources Canada. Therefore, recovery of unconventional crude oil sources such as the Alberta oil sands produces large volumes of OSPW which must be stored on-site due to a zero-release policy in the Alberta Environmental Protection and Enhancement Act of 1993. Research examining OSPW toxicity has focused on the naphthenic acid (NA) containing organic fraction (OSPW-OF), indicating it as the primary toxic component. Comparatively, little is known about the contribution of the inorganic fraction (OSPW-IF) to whole effluent toxicity.

Using an *in vitro* bio-indicator system to compare whole OSPW and OSPW-OF, we showed that whole OSPW was significantly more toxic than OSPW-OF at NA concentrations from 10-18 mg/L. These findings suggest that at NA concentrations reported in tailings ponds (<20 mg/L), inorganic constituents and/or interactions between OSPW-OF and OSPW-IF may significantly contribute to whole OSPW toxicity. Using various cell-based assays we evaluated the relative contributions of OSPW-OF and OSPW-IF exposures, alone or combined, on immunotoxicity in mammalian macrophage cells. This work suggests that constituents present in OSPW-IF may contribute to the overall immunotoxicity of whole OSPW.

14. Microplastic Pollution in the Urbanized North Saskatchewan River

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MacEwan University

There is growing interest in understanding sources of microplastics in freshwater environments. Microplastics are plastics that are < 5mm in size, comprised of fibres, fragments, beads, and films. This project investigates microplastic occurrences and potential point sources within the urban North Saskatchewan River in Edmonton, Alberta. Water samples were collected with 53µm plankton nets at 7 sites along this river in June 2017, upstream and downstream from Goldbar Wastewater Treatment Plant (WWTP), a likely point source. Following sampling, $ZnCl_2$ density flotation and wet peroxide oxidation were used to isolate microplastics from co-collected material. After sieving the material into five size classes, visual microscopy revealed the presence of microplastics in the samples, with a range of types and colours. The findings suggest that Goldbar WWTP is likely not a point source for microplastic

pollution in this urban river, contrasting similar studies that have generally found the opposite trend. Fragments were the primary plastic type recovered across all sites, yet differences in the proportions of microplastic types exist between larger and smaller size classes. For instance, the 500µm - 1mm size class contained more fibres upstream and more fragments downstream of Goldbar WWTP. The 53 – 125µm size class also contained a higher proportion of beads compared to the 500µm – 1mm size class. This study reveals that urban populations influence microplastic contamination in various ways, and the data will provide a valuable baseline for future monitoring studies.

15. Salts & Oil - Assessing the Environmental Toxicity of Hydraulic Fracturing Flowback and Produced Water

Yuhe He[1], Tamzin Blewett[1], Erik Folkerts[1], Perrine Delompre[1], Shannon Flynn[2], Yifeng Zhang[3], Chenxin Sun[3], Danial Alessi[2], Jonathan Martin[3], Greg Goss[1]
University of Alberta

The use of hydraulic fracturing (HF) has emerged as a major method of recovering unconventional oil and gas resources in the United States and Canada. Despite the rapid development of HF practice, many environmental questions remain. One of the major issues is the risk of spills of hydraulic fracturing flowback and produced water (HF-FPW). HF-FPW is the wastewater returns to the surface after well fracturing simulation. The environmental risk of HF-FPW is complicated by its large volume as well as the combined complexity of organic and inorganic constituents in HF fluids and deep formation water. We are the first research group characterizing the salts/solids, organic and toxicological signatures in real HF-FPW samples from the Duvernay Formation, Alberta, Canada. Huge amounts of salts and numerous unknown dissolved polar organics were detected in HF-FPW samples. Acute and sub-lethal toxicity were also assessed by using a variety of aquatic species models. Our research program demonstrates that chemical and toxicological profiling of real HF-FPW sample presents great challenges for assessing the potential risks and impacts posed by HF-FPW spills. Future study will be focus on the toxic mechanism(s) of HF-FPW on freshwater species in spatial, temporal and fractional manners to provide essential information in establishing monitoring, treatment, and remediation protocols for HF-FPW.