Photodegradation of Pharmaceuticals in Aquatic Environments: Defining our Current Understanding and Identifying Knowledge Gaps

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This presentation summarizes a critical assessment of the state and quality of knowledge around the aquatic photochemistry of human-, veterinary-, and agricultural-use pharmaceuticals in laboratory experiments and field observations. We used a weight-of-evidence-style approach to identify knowledge gaps in the literature regarding the photolytic fate of pharmaceuticals in surface waters. A special sub-focus was on characterizing the ability of laboratory studies to predict fate under field conditions. A standardized scoring rubric was used to assess the quality of the methodology and experimental design of relevant laboratory and field studies. The rubric provides an objective and scientifically-defensible set of metrics for assessing the quality of a study, and will provide a benchmark for the design of future photolytic fate studies.

In general, those drugs that were most studied also had relatively good quality data. The four pharmaceuticals studied experimentally at least ten times in the literature (carbamazepine, diclofenac, sulfamethoxazole, propranolol) had average total scores (lab and field combined) of ≥29; considered as good quality. A major recommendation from this work is the development of a method guideline, based on this rubric, for conducting and reporting on photochemical studies that would produce consistent and reliable data for quantitative comparison across studies. Furthermore, an emphasis should be placed on conducting more dual-fate studies involving controlled photolysis experiments in natural sunlight, and whole system fate studies in either natural or artificial systems. This approach would provide accurate data describing the actual contribution of photolysis to the overall fate of pharmaceuticals in the environment.

Key Words: photodegradation, pharmaceuticals, weight of evidence, critical review

Reducing Contaminants and Toxicity in Rural Wastewater Effluent Using New Subsurface Treatment Technology

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The ability of a sub-surface treatment filtration system to remove nutrients, a suite of thirty-nine organic contaminants, and antibiotic resistant gene (ARG)-bearing organisms, and to attenuate acute toxicity of wastewater lagoon effluents in the field was assessed. Significant removal was observed for nutrients between the conventional primary and secondary sewage lagoons, with further typical attenuation of 59% and 50% of ammonia and total phosphorus (TP), respectively, within the filter. Effluent concentrations of ammonia ranged from 0.4 to 2.6 mg/L and concentrations of TP from 1 to 4.1 mg/L, with decreasing toxicity from primary to secondary lagoons, and increasing favorable conditions for microbial growth based on Microtox® assays. Most monitored organic micropollutants were efficiently removed between the primary and secondary lagoons. However, in general, little measurable attenuation occurred within the filter for estrogenic compounds (e.g., 17α-ethinylestradiol); β-blockers (e.g., metoprolol); antidepressants (e.g., fluoxetine--Prozac); antibacterial agents (e.g., triclosan), non-steroidal anti-inflammatory drugs (e.g., diclofenac); lipid regulators (e.g., clofibric acid); and macrolide (e.g., clarithromycin) and sulfonamide (e.g., sulfamethazine) antibiotics. This lack of removal was likely due to minimal hydraulic residence time within the filter (~6 hr). In order to assess abundance, distribution, and removal of ARGs, substrates were deployed for biofilms and water were analyzed. Genes associated with nitrogen transformations were also quantified to corroborate nutrient removal efficiencies. Antibiotic resistance genes were efficiently filtered from wastewaters by the filter, which served as a repository for these genes. These results suggest that the constructed sub-surface treatment filtration system can provide a low cost, low maintenance, and effective means to provide a reduction in nutrient loading and ARG-bearing organisms.

Key Words: Wastewater lagoons, filtration, pharmaceuticals, antibiotic resistance genes

The Reliability of Sucralose as a Wastewater Contamination Tracer

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Sucralose (Splenda®) is an artificial sweetener that is difficult to metabolize, and is thus excreted unchanged by consumers. Sucralose is considered suitable as a wastewater tracer due to its high water solubility, chemical stability, sole environmental input via human excretion, and hence its presence in the environment via wastewater. Our work shows the rapid disappearance of sucralose from shallow freshwater wetland systems through exposure experiments on 1,500L wetland mesocosms containing macrophytes (e.g. *Typha* spp.) to varying levels of sucralose (1, 100, 1000µgL^{-1}) in triplicate. Sucralose was removed from the water column with pseudo-first order rate constants of 0.053 $\pm$ 0.013 (σ), 0.047 $\pm$ 0.021, and 0.043 $\pm$ 0.019 respectively. Sucralose was found in *Typha* at concentrations up to 0.8µg/g and 0.02µg/g in leaves and shoots, respectively, and in sediments at concentrations of 0.4µg/g. There are no other known removal processes for sucralose at this time. These results indicate that sucralose was likely removed from the water column, at least in part, by root uptake of sucralose in sediment porewaters by macrophytes, followed by translocation of this sugar mimic in plants. Therefore, sucralose cannot be used to quantitatively measure wastewater contamination due to its uptake by *Typha* in wetland environments, although it can qualitatively indicate the presence of wastewater.

Key Words: sucralose, wastewater, *Typha* spp.

Reconstructing Spatial and Temporal Changes in Phytoplankton Abundance in a Narrow River-valley Reservoir

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Paleolimnology can be used to reconstruct long-term environmental trends in reservoirs as well as lakes. Although lakes and reservoirs are similar in some ways, reservoirs tend to be more spatially heterogeneous and have distinctive physical, chemical and biological spatial gradients. Using Lake Diefenbaker, in Saskatchewan, Canada, as a test case, this study reconstructed long-term trends in primary productivity within the context of these spatial gradients. Cores of sediment were collected from the main channel along the longitudinal axis of the reservoir. These cores were sectioned vertically into 1-cm increments and analyzed for an array of fossil pigments representative of a diversity of taxonomic groups. Trends in pigment concentrations in recent surface sediments (0-1 cm) suggest an increase in algal productivity temporally with distance down-reservoir, with greatest primary production in mid-reservoir region. Concentrations of pigments in vertical profiles of down-reservoir sediments suggest increasing primary productivity over time. An increasing trend of the pigment myxoxanthophyll, produced by filamentous and colonial cyanobacteria in the areas furthest down-reservoir, suggests increasing production of potentially harmful cyanobacteria. Overall, these results demonstrate the usefulness of paleolimnology in reconstructing long-term trends in reservoirs and the importance of investigating both spatial and temporal variability when assessing trophic status of a dammed river-valley reservoir.

Key Words: sediment, pigments, reservoir, cyanobacteria

Platform Session

Three Novel Brominated Flame Retardants Affect Fecundity and Transcript Profiles of the HPGL-axis in Japanese Medaka

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The novel brominated flame retardants (NBFRs), Bis(2-ethylhexyl)-2,3,4,5-tetrabromophtalate (TBPH), 2-ethylhexyl-2,3,4,5 tetrabromobenzoate (TBB), and 1,2,5,6 tetrabromocyclooctane (TBCO) are components of flame retardant mixtures including Firemaster 550 and Saytex BC-48. Despite the detection of these NBFRs in environmental abiotic and biotic matrices, little is known about their toxic effects in aquatic organisms. Previous *in vitro* assays have reported potentials of these three NBFRs to modulate endocrine signaling. To investigate effects on fecundity and endocrine function, a 21-day short term fish reproductive assay was adapted for Japanese Medaka (*Oryzias latipes*). Sixteen fish, 8 male and 8 female, were fed high (15 µg/g food) or low (1.5 µg/g food) doses of TBCO, and high (1500:1500 µg/g food) or low (150:150 µg/g food) doses of a TBPH/TBB mixture. Cumulative production of eggs was measured as a marker of fecundity while transcriptional profiles of 35 genes along the hypothalamus-pituitary-gonadal-liver axis were also assessed to determine mechanisms of effects. Cumulative fecundity was inhibited in fish exposed to either toxicant group and significant changes in transcript profiles of the HPGL axis were observed. Genes representing biomarkers of estrogenic effect including vitellogenin and choriogenin were significantly up-regulated in fish exposed to TBCO but not those exposed to the TBPH/TBB mixture.

Key Words: Novel Brominated Flame Retardants

Platform Session

Predicting the Sensitivity of Endangered Sturgeons to Dioxin-like Compounds: Molecular Investigation into the Aryl Hydrocarbon Receptor Pathway

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Worldwide, populations of sturgeons are endangered. However, assessments of risk to pollution are hampered by lack of knowledge about their sensitivity to chemicals of concern, such as dioxin-like compounds (DLCs). DLCs elicit their toxic action through activation of the aryl hydrocarbon receptor (AhR). To allow more objective risk assessments, the objectives of this study were to: 1) characterize the relative differences in sensitivity to DLCs between two species of endangered sturgeon (white sturgeon (WS) and lake sturgeon (LS)), and 2) develop methods to predict the relative sensitivity of other sturgeons to DLCs based upon differences in mechanisms of toxicity. WS were found to have 6-fold greater response compared to LS based on up-regulation of CYP1A transcript. Since effects of exposure to DLCs are known to be driven by activation of the AhR, the AhR1 and AhR2 of WS and LS were sequenced and characterized in an attempt to explain the observed differences *in vivo*. WS and LS AhR1s had similar sensitivity to 2,3,7,8-TCDD *in vitro* by use of the LRG assay with transfected COS-7 cells, while WS AhR2 had an EC_{50} 10-fold less than LS AhR2. Relative differences in response between WS and LS *in vivo* and with regard to AhR2 activation *in vitro* appear similar indicating that AhR2 might drive differences in sensitivity to DLCs among these sturgeons.

Key Words: fish, risk assessment, LRG assay, in vitro

A Comparative Study of the Toxicities of Ionic and Nanoparticulate Silver in Zebrafish (*Danio Rerio*)

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Materials in the nanoscale may have enhanced and/or novel properties compared to particles of a larger size and there is thus a requirement to establish if this also confers novel or increased risk to aquatic organisms. For many metallic nanoparticles (NPs) there is also the need to discriminate between effects attributable to the NP, and effects mediated by metal ions that are released from the NP surface when in aqueous suspension. At present, environmental metals legislation for freshwaters in North America are derived from data of organisms' responses to dissolved metals; NPs are not explicitly considered. In this study, the toxicities of silver NPs (Ag-NPs, 50.7 ± 25.9 nm) and ionic silver (Ag^+, as $AgNO_3$) to zebrafish embryos were compared. In acute toxicity tests, calculated LC_{50} values for Ag^+ were several orders of magnitude lower than for Ag-NPs. However, the mechanisms of toxicity appear similar for both Ag^+ and Ag-NPs. Exposure to ½ LC_{50} concentrations of both materials resulted in comparable inhibitions of $^{22}Na^+$ uptake. Exposure to both forms of Ag also induced increased expression of metallothionein in embryos. These preliminary data suggest the mechanisms of toxicity of Ag-NPs and Ag^+ in zebrafish may be the same and the toxicity of Ag-NPs is likely driven by dissolution of Ag^+ from the particle surface.

Key Words: silver nanoparticles, zebrafish, sodium homeostasis, toxicity

Metal-oxide Nanoparticles Alter Receptor-mediated Signal Transduction and the Functional Capacity of Innate Immune Cells

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The toxicological response of the vertebrate immune system to nanomaterials (NM) has not been examined to a great extent despite its importance to overall organism health and the successful use of NMs in consumer products. Organisms may internalize NMs via inadvertent environmental exposure when products are disposed, leading to exposure of the immune system. Current data indicate that NMs can induce both immunosuppression and immunoactivation in fish and mammals, and we have recently shown that some NMs may inhibit degranulation (the release of granular inflammatory mediators to combat infections) of RBL-2H3 cells, a rat mast cell line. Thus, there is concern that NMs could promote and/or suppress inflammatory responses. However, to date there has been limited comprehensive research dedicated to understanding these critical immunological interactions, and especially the mechanisms that govern these observations. Therefore, the aim of this study was to determine whether decreases in RBL-2H3 degranulation were related to changes in receptor binding affinities for IgE antibodies, using flow cytometry, and to changes in signal transduction of intracellular kinases that mediate degranulation, using Western Blotting techniques. The effects on cell viability were also determined. Cells were exposed to polymer-coated metal-oxide NMs (TiO_2, CeO_2) at various concentrations for up to 24 hours in a series of experiments. Our results show significant suppression of the degranulatory response that is at least partially mediated by changes in the receptor binding capacity and signal transduction kinase activity of the immune cells.

Key Words: immune, toxicology, nanomaterials, degranulation

Platform Session

Effect of a Metal Mixture on the Olfactory System of Rainbow Trout (*Oncorhynchus mykiss*)

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Contamination of fish with individual metals decreases their ability to perceive their environment through olfaction. Little work has been done, however, to determine the effect of complex metal mixtures on olfactory function in fish. In this study we exposed rainbow trout (*Oncorhyncuhs mykiss*) to one of five treatments; cadmium (Cd), copper (Cu), nickel (Ni), zinc (Zn), or a mixture of all four metals at current guideline concentrations. Cadmium by itself induced olfactory dysfunction, while exposure with Cu, Ni, Zn, or the mixture had no effect. Binary mixtures of Cd with Cu, Ni, or Zn demonstrated that Ni and Zn, but not Cu, protected against Cd-induced olfactory dysfunction. Two possible mechanisms of protection from Cd-induced olfactory dysfunction by Ni and Zn were then investigated. First, co-exposures of Cd with sodium, magnesium, or calcium were performed to test whether or not this protection was related to the electrolyte-mimicking properties of the metals in question. Second, quantitative PCR experiments to measure the gene expression of metallothionein and catalase under Cd and a mixture exposure were performed. Neither of these possible mechanisms appears to be conferring protection, leading to the conclusion that the protective effect of Ni and Zn is most likely due to competition with Cd for intracellular binding sites associated with toxicity. By understanding how metal mixtures affect olfaction we can better predict safe metal concentrations in waterways.

Key Words: olfaction, metal, mixture

In-Channel Beaver Impoundments Increase Availability of Methylmercury to Stream Food Webs

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Beaver (*Castor* sp.) are ecosystem engineers, important modifiers of freshwater ecosystems. Their impoundments alter the flow of materials through streams, thus potentially increasing primary and secondary productivity but also the availability of methyl mercury (MeHg) to downstream food webs. MeHg is toxic to wildlife and humans; therefore quantifying its biomagnification potential in aquatic food webs is critical. This study examines food web-available MeHg above and below 15 in-channel beaver impoundments in the southern Canadian Rockies. While nutrients, algal biomass and invertebrate standing stock were not significantly elevated below ponds, there was a consistent doubling of MeHg concentrations in water, periphyton and invertebrates downstream relative to upstream. Food web biomagnification, measured by the slope of the δ^{15}N-logMeHg relationship, was typical (slope=0.18) of temperate freshwater systems and did not vary between upstream and downstream sites. This suggests that beaver impoundments increase the availability and subsequent uptake of MeHg by basal food web organisms even if they have no effect on resource availability. The findings present important implications for research and management of mercury in stream drainage networks where beavers are present.

Key Words: mercury, stream ecology, ecosystem engineers

What does your evidence weigh?

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Some pesticide risk assessments are based on an extensive, and ever growing, relevant body of scientific literature leading to the challenge of sorting out the strong studies from the weak. A quantitative weight of evidence (WoE) approach has been created to help place the results of studies in their appropriate context. The methodology involves an assessment of 1) effects related to pre-selected outcome(s) relevant to whole animal performance and 2) the strength of the experimental methods employed. Each response examined is considered in relation to statistical significance, consistency of the response, relevance to apical endpoints (e.g., survival, growth, reproduction, and development), and the underlying plausibility of the mechanisms leading to the observed effect. We characterize the strength of the experimental methods by scoring the hypothesis being tested and the experimental design, the use of GLP and QA/QC, number of concentrations used, and the inclusion of realistic and upper-bound values in the range of concentrations tested. The means of the scores of these two assessments are then used to summarize and weigh the evidence for the contribution of the response of interest to significant changes in the environment. The WoE approach developed provides a transparent, reproducible and robust framework that can be used to assist the decision-making process when assessing pesticides, and helps to identify knowledge-gaps for future studies. The approach will be illustrated using atrazine and responses related to fish, reptiles and amphibians.

Key Words: risk assessment, pesticides, weight of evidence

Factors Influencing Fate and Persistence of Neonicotinoid Insecticides in Prairie Wetlands

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Neonicotinoids are commonly used as seed treatments on Canada's major prairie crops such as canola, cereals and pulses. Neonicotinoids frequently drain into agricultural surface waters, but their ultimate fate in wetland ecosystems is largely unknown. In a two-year survey of wetlands in central Saskatchewan, neonicotinoid detection rates varied between 16% (fall 2012) and 91% (spring 2013). Neonicotinoid concentrations also varied seasonally by several orders of magnitude, with means ranging between 4.0 ng/L (fall 2012) and 167 ng/L (summer 2013), with maximum concentrations as high as 3110 ng/L (summer 2012). We hypothesize that high spring (pre-seeding) detection rates may be a result of long-term persistence, repeated additions or transport influenced by interactions between aged soil particulate and snowmelt. In summer, when peak concentrations occur, it is currently unclear why certain biologically-similar wetlands are particularly susceptible to neonicotinoid contamination. We address these questions through detailed studies of 250 ponds where we collected over 40 wetland (e.g. plant zonation, surficial cover) and landscape (e.g. crop, wetland situation) variables as part of a novel rapid wetland assessment system. Preliminary results indicate surrounding land use and wetland water permanency may influence neonicotinoid concentration. Models will be used to identify features that influence the fate, transport and persistence of neonicotinoids in Prairie wetlands. This work has important implications for conservation of wetlands and could potentially inform efforts to mitigate pesticide effects on wetland dependant organisms.

Key Words: neonicotinoids, insecticides, agricultural wetlands, landscape ecology

Platform Session

Effects of Neonicotinoid Insecticides on Aquatic Insect Emergence in Prairie Wetlands

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Prairie wetlands are highly productive ecosystems and provide a critical energy link between aquatic and terrestrial environments in the Prairie Pothole Region (PPR). Recent evidence suggests wetlands within the Canadian PPR associated with arable land are susceptible to repeated or persistent low-level neonicotinoid contamination via run- off. Neonicotinoids are a widely used newer class of nicotine based systemic insecticides favored for their high water solubility, various application methods, and high toxicity to invertebrate pests. As a consequence, non-target aquatic insects may be at risk to lethal and sub-lethal effects particularly from chronic exposure. This work focuses on using chronic, full life cycle tests using more sensitive endpoints such as emergence at environmentally relevant concentrations. Here, I will describe results from field and laboratory studies examining the individual and population level effects of neonicotinoid insecticides on aquatic insect emergence patterns.

Key Words: insecticide, macroinvertebrate, wetland, chronic toxicity

Effects of Agricultural Intensification and Neonicotinoid Insecticides on Tree swallow (*Tachycineta bicolor*) Diet and Reproduction

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Over the past 30-40 years abundances of many species of aerial insectivorous birds (i.e., birds that feed on flying insects) have been declining. One hypothesis proposed to explain this trend is lower food availability due to agriculture intensification, including increased pesticide applications. Neonicotinoids are a group of insecticides widely used as seed treatments across the prairies as an effective way of controlling pest insects. However, evidence shows that neonicotinoids are highly toxic to non-target insects which are important food sources to many aerial insectivores. Our study evaluates whether agricultural intensification (1) affects reproductive success and nestling body condition in an aerial insectivore, the tree swallow and (2) alters nestling and adult diets through potential spatiotemporal changes in insect availability. Higher neonicotinoid detections and concentrations occurred in areas dominated by cropland relative to areas of grassland and pasture. Abundance and biomass of aerial insects tended to be lower on sites with higher agricultural intensification. Tree swallows breeding on study sites with low percent crop cover within the swallow's foraging range had larger clutch sizes and nestlings in better body condition. However, nestling and adult diets did not differ among study sites, with swallows showing a consistently strong reliance on aquatic dipteran prey, one of the most sensitive groups of insects to the pesticide. Tree swallows are sensitive indicators of agricultural intensification and results will aid decisions about future research and management of declining aerial insectivore populations.

Key Words: agricultural intensification, diet, neonicotinoids, tree swallows

Developmental Exposure to Aroclor 1254 Alters Migratory Behaviour in Juvenile European Starlings (*Sturnus Vulgaris*)

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Onset of migratory restlessness, orientation, and navigation during avian migration are directly under neurological and hormonal control. Birds exposed to endocrine disrupting chemicals during development may exhibit neurological changes affecting migratory behaviour. We evaluated how early exposure to a low dose PCB mixture and known thyroid-hormone disruptor, Aroclor 1254, could cause latent effects in juvenile European starlings (*Sturnus vulgaris*). Eighty-four birds were orally administered 0, 0.35, 0.70, or 1.05 µg Aroclor 1254/g-bw from 1 to 18 days post-hatch. At 4 months, birds were subsequently exposed to a 6-week captive photoperiod shift simulating an autumn migration. Nestlings exhibited no significant differences in growth measures except for wing asymmetry. During the simulated migration period, significant increases were observed in mass, fat, and moult score over time for all treatments, combined with significant increases in activity suggesting migratory readiness was induced. At 12L:12D, control birds showed a directional preference for 155.95° (South-southeast), while birds treated with 1.05 µg Aroclor 1254 exhibited a random distribution, indicating no orientation ability. High dose birds instead showed delayed directional preferences for 197.48° (South-southwest) under 10L:14D, concomitant with delays in moult. These findings link alterations in avian behaviour to contaminant-specific mechanisms. Subtle contaminant induced changes during development could give rise to larger-scale effects, including changes in migratory activity and orientation behaviour, which may partly explain observed global declines in migratory species.

Key Words: PCB, orientation, endocrine disruption

Role of Human Multidrug Resistance Protein 4 (MRP4) in Arsenic Transport

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Chronic arsenic toxicity is a human health hazard affecting 160 million individuals worldwide (including Canada) leading to multi-organ cancers. Exposure occurs mainly through contaminated groundwater and crops, arsenic containing pesticides, arsenic utilizing industries and from pharmacologic sources. Inorganic arsenic is extensively methylated and glutathione conjugated in the human liver, generating metabolites with different toxicity profiles, and is excreted mainly through urine. Little is known about the mechanism(s) by which arsenic metabolites are transported from liver to blood and kidney and subsequently eliminated through urine.

In the present study, we demonstrate that MRP4 is responsible for the transport of two arsenic metabolites, the diglutathione conjugate of monomethylarsonous acid ($MMA(GS)_2$) and dimethylarsinic acid (DMA^V) in an ATP dependent manner. Both these molecules are high affinity ($K_{0.5}$ of 0.70 ± 0.16 µM and 0.22 ± 0.15 µM, respectively) substrates for MRP4, and the transport is osmotically sensitive and positively allosteric in nature. DMA^V transport was also pH dependent showing higher affinity and capacity at pH 5.50 ($K_{0.5}$ of 0.073 ± 0.018 µM, V_{max} of 50 ± 2.3 pmol mg^{-1} protein min^{-1}). MRP4 is the first experimentally demonstrated transporter of DMA^V, the predominant form of urinary arsenic, and could play a pivotal role in regulating arsenic toxicity, susceptibility and carcinogenicity.

Key Words: MRP4, carcinogenicity, $MMA(GS)_2$, DMA^V

Selenium vs. Arsenic: A Whole Food Approach to Combat Arsenic-related Cardiovascular Disease

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Cardiovascular disease (CVD) is the major cause of death worldwide, and arsenic intake mainly through drinking water is a well-known risk factor for CVD.
We tested the potential of high-selenium (Se) lentils from Saskatchewan in altering the progression of As-triggered atherosclerosis. Mice exposed to 200ppb As in their drinking water and control mice received one of three lentil diets: Se-deficient (0.009 mg/kg), Se-adequate (0.16 mg/kg), or Se-fortified (0.3 mg/kg). After 13 weeks, atherosclerotic lesions in the aortic arch and sinus were significantly increased in As-exposed mice compared to controls, when comparing mice on Se-deficient and Se–adequate diets. Lesion formation was substantially reduced in As-exposed mice on the Se-fortified diet. Plaque cell content changed to a less stable composition (more likely to rupture and cause emboli) in the Se-deficient mice of both As and control group. Favorable serum lipid levels were observed in mice on the Se-adequate diets in both the control and As-exposed group, yet in the latter lipid levels were further improved by the high-Se diet. Future studies should examine, if dietary Se supplementation could suppress atherogenesis in As-exposed human populations.

Key Words: arsenic poisoning, selenium, lentils, atherosclerosis