Proceedings of the 32nd Annual Aquatic Toxicity Workshop: October 3 to 5, 2005, Waterloo, Ontario

Comptes rendus du 32ième atelier annuel sur la toxicité aquatique: du 3 au 5 octobre 2005, Waterloo, Ontario

Editors/Éditeurs

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Rapport technique canadien des sciences halieutiques et aquatiques 2617

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Rapport technique canadien des sciences halieutiques et aquatiques

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Preface/Preface

The 32nd Annual Aquatic Toxicity Workshop was held at the Waterloo Conference Centre in Waterloo, Ontario, October 3 to 5, 2005. The Workshop included 3 plenary presentations, 147 platform and 64 poster papers. Total attendance was 379.

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

L' 32ième atelier annuel sur la toxicité a eu lieu au Centre de Conférence de Waterloo à Waterloo, Ontario, du 3 au 5 october 2005. Le atelier a donné lieu a 3 communication lors de séances plénières, 147 exposés d'invités d'honneur 64 communications par affichage. 379 personnes ont assisté au atelier.

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

Editors comments/Remarques des editeurs

This volume contains papers, abstracts or extended abstracts of all presentations at the Workshop. An author index and list of participants are also included. The papers and abstract 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 or rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Ces comptes rendus sont publiés en deux volumes, en raison de leur longeur, ils renferment le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs et une liste des participants sont 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 lonueur et la forme des communications varient parce que ces derniéres sont pour la plupart publiées intégralement. On est prié de communiquer directement avec les auteurs pour faire des remarques sur le travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont celles des conférenciers; elle ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

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Table of contents/Tables des matiére

Plenary/Plénière

Contributed papers/Documents contribués

*(PL) Platform paper/Présentation orale, (PO) Poster paper/Présentation affichée.

Plenary/Plénière

Reflections of a pickle crock ecologist
Dr. John B. Sprague
Sprague and Associates, Saltspring Island, Vancouver, BC.

Rick Playle: fish physiologist, exotoxicologist, modeler and superb human being
Dr. Christopher M. Wood
Department of Biology, McMaster University, Hamilton, ON.

Ecological and human risk assessment of the use of herbicides in the control of illicit crops in Latin America
Dr. Keith R. Solomon
Department of Environmental Biology, University of Guelph, Guelph, ON.

Contributed papers/Documents contribués

Great Lakes science policy/Politique scientifique des Grand lacs
Session chair/Président: Julie Schroeder

Great Lakes aquatic toxicology and the International Joint Commission: a forty-year retrospective. M. Gilbertson. International Joint Commission (retired), Windsor, ON.

A radiodated sediment core from any part of the Great Lakes can show the social history of modernization within the basin during the past century. The increases in releases of persistent toxic substances, as chemical manufacturing grew, are written in the undisturbed profundal sediments. These increases resulted in widespread contamination and extirpation of several species of fish and wildlife and serious effects on developmental processes in human infants. The International Joint Commission was sent a reference in 1964 to report on pollution of the lower Great Lakes and the interconnecting channels. Their 1969 reports coincided with the widespread "struggle" known as the New Social Movements. Governments legitimized the outrage over environmental degradation through legislative reform and through the 1972 signing of a *Great Lakes Water Quality Agreement* (GLWQA). This "resistance" to corporate power resulted in spectacular declines in the concentrations of persistent toxic substances in the 1970s and the return of several extirpated wildlife species. The neo-liberal agenda of the 1980s resulted in the "capture" of the environmental reform processes. There was a brief successful renewal of "resistance", lead by non-government organizations, which was focused on the International Joint Commission between 1989 and 1992. But with the election of President Clinton, industry representatives persuaded the White House to appoint U.S. Commissioners "who were not as green as the current bunch". Since the environmental reforms have been "recaptured", little progress has been made in implementing the GLWQA. Several fish species have not yet recovered from the ravages of persistent toxic substances from the past century and effects are still occurring on humans. New persistent toxic substances have replaced those proscribed by law and their concentrations are increasing. The question posed is whether a new period of "struggle", characterized by widespread student unrest, is required to bring about new institutional reforms for environmental restoration and protection.

Federal and provincial efforts to reduce harmful pollutants in the Great Lakes Basin – the *Canada-Ontario Agreement.* J. Schroeder[1] and T. Tseng[2]. [1]Ontario Ministry of the Environment, Laboratory Services Branch, Etobicoke, ON; and [2]Environment Canada, Toxics Prevention, Downsview, ON.

Under Annex 2 of the 2002 *Canada-Ontario Agreement* (COA) Respecting the Great Lakes Basin Ecosystem, the federal and provincial governments have committed to the virtual elimination of persistent toxic substances such as mercury, polychlorinated biphenyls and dioxins, as well as the reduction of other substances of concern. Implementation of the Annex commitments is coordinated by Environment Canada and the Ontario Ministry of the Environment. Projects within Annex 2 include those focusing on municipal wastewater treatment plant discharges, voluntary reductions by priority industrial sectors, and reductions in air emissions. Additionally, an important goal of the Agreement is the enhancement of knowledge of priority and emerging substances of concern through research of their sources and fate as well as their impacts on human and environmental health. Results of this research will be summarized to facilitate the development of priorities for the next Canada-Ontario Agreement, once the current agreement ends in 2007. Activities related to the research and reduction of harmful pollutants in the Great Lakes Basin will be discussed as well challenges associated with meeting established targets.

Cataraqui River Project Trackdown: a pilot study for tracking polychlorinated biphenyls in Lake Ontario tributaries. N.B. Benoit[1], A.E. Dove[2] and W. Herrick[3]. [1]Ontario Ministry of the Environment, Environmental Monitoring and Reporting Branch, Etobicoke, ON; [2]Environment Canada, Ecosystem Health Division, Burlington, ON; and [3]Ontario Ministry of the Environment, Eastern Region, Kingston, ON.

As part of a commitment to the Lakewide Management Annex of the *Canada-Ontario Agreement* to conduct watershed investigations focused on identifying sources of Lake Management Plan critical pollutants, the Ontario Ministry of the Environment and Environment Canada implemented a joint pilot study in three Lake Ontario Tributaries (Twelve-Mile Creek, Etobicoke Creek, Cataraqui River). Cataraqui River was selected as one of the "PCB Source Trackdown" pilot studies because previous sampling efforts indicated elevated PCB levels in fish and sediments in its downstream reaches. The purpose of the "PCB Source Trackdown" pilot study was to determine if it was possible to identify ongoing and locally controllable sources of PCBs to Lake Ontario tributaries. Trackdown efforts included the sampling of sediments, water, biota (caged mussels, young-of-year fish, benthic invertebrates), semi-permeable membrane devices (SPMDs) and sediment bioassays. Two local anomalies were identified in sediment in the Cataraqui River inner harbour. Sediment toxicity tests supported results from biota and SPMDs that suggested that PCBs were bioavailable and, that sediments from the sites were a source of PCB to exposed organisms. Groundwater monitoring indicated no major ongoing source of PCBs to the inner harbour, suggesting contamination is likely historical. Localized dredging was conducted to remove contaminated sediment adjacent to Emma Martin Park. Monitoring is planned to assess whether sources have been controlled. Source trackdown was most effective when several matrices were used concurrently to provide a weight-of-evidence approach in determining the potential presence of contaminants; this approach has been implemented in other studies.

Cataraqui River Project Trackdown: assessing potential human health risks from recreational exposure to contaminated sediment. P.G. Welsh, M. Pagliarulo and D. Manca. Ontario Ministry of the Environment, Environmental Standards Branch, Toronto, ON.

Project Trackdown for the Cataraqui River is one of several pilot studies to address the Lake

Ontario Lake Management Plan commitment to reduce sources of critical pollutants to the Great Lakes. Polychlorinated biphenyls (PCBs) were chosen as the critical pollutant for this project. Sediment and aquatic biota from two areas of the Cataraqui River were found to have elevated PCB concentrations. The maximum PCB concentration in sediment was between two docks used for boat access by rowers associated with a local rowing club. To assess potential human health concerns from recreational exposure to contaminated sediment at this site, a screening-level health risk assessment was conducted. PCBs, Sb, As, Be, Fe, Pb, and benzo(a)pyrene were identified as contaminants of concern (COCs). Exposure was estimated for model receptors, representing rowers and summer camp members. Dermal contact with contaminated sediment and incidental ingestion of sediment suspended in water were identified as relevant exposure pathways. Predicted exposure to COCs from dermal contact and ingestion of contaminated sediment was relatively low and unlikely to result in any adverse health effects (HQ < 1; negligible excess cancer risk). This study supports commitments under the *Canada-Ontario Agreement* to enhance knowledge of the occurrence, fate, and impact of harmful pollutants on human and environmental health in the Great Lakes.

Occurrence of pesticides in the Don and Humber River watersheds (1998 to 2002). T. Fletcher[1], J. Struger[2] and G. Gris[3]. [1]Ontario Ministry of the Environment, Standards Development Branch, Toronto, ON; [2]Environment Canada, Ecosystem Health Division, Burlington, ON; and [3]City of Toronto, Works and Emergency Services Department, Toronto, ON.

In 1998, a study of the Don and Humber River watersheds was initiated to determine the concentrations of pesticides in surface water, with a particular focus on those used in lawn care. Water samples were collected and analyzed for up to 152 pesticide active ingredients and eight metabolites, including phenoxy acid herbicides organophosphorus insecticides, and other pesticides associated with lawn care use. Samples were collected from several sites in the Don and Humber River watersheds during base flow and rainfall events from 1998 through 2002. Eleven pesticides and one metabolite were detected in surface waters of the Don and Humber Rivers or their tributaries. Water quality criteria (CCME Canadian Water Quality Guideline or Ontario Provincial Water Quality Objective) were exceeded for four pesticides. Except for diazinon, these exceedences were infrequent. Diazinon exceeded the Provincial Water Quality Objective for the Protection of Aquatic Life in 27% of the samples taken. For the other three pesticides (atrazine, carbofuran, and chlorpyrifos) exceedence of a water quality criteria occurred in less than 1% of the samples. This would suggest that occurrence of these pesticides are unlikely to have an impact on the health of aquatic organisms.

The challenge of developing Water Quality Criteria for emerging compounds. T. Fletcher[1] and D.J. Spry[2]. [1]Ontario Ministry of the Environment, Standards Development Branch, Toronto, ON; and [2]Environment Canada, National Guidelines and Standards Office, Gatineau, QC.

Contaminants that impair water quality are a major environmental concern. Regulating the discharge of contaminants, and assessing the consequence of their discharge are important components of water policies both at the provincial and federal levels of government in Canada. Many jurisdictions use a defined level of water quality to provide a baseline for assessing the quality of surface waters and which can act as a surrogate measure for ecosystem health. Development of these water quality criteria (e.g., Canadian Water Quality Guidelines, Provincial Water Quality Objectives) typically requires a minimum number of published, good quality toxicity data from a wide variety of organisms that are representative of the aquatic life within the waters of that jurisdiction. Typically, these data reflect the "Big Three" significant endpoints that can be assessed; survival, reproduction and growth. The "discovery" of new contaminants in the environment often receives significant press coverage and government scientists are under pressure to develop new water quality criteria in order

to assess the environmental implications of these contaminants. There are two significant challenges to do this; the lack of a large enough toxicity database for the contaminant and an increasing emphasis on other, more sensitive, endpoints (e.g., endocrinology, organ morphology) which are typically not useful for the development of water quality criteria. Possible solutions to this problem will be discussed.

Thinking outside the tox: indirect effects in Ecological Risk Assessment (ERA). R.N. Hull. Cantox Environmental Association, Inc., Mississauga, ON.

Direct toxic effects (survival, growth or reproduction) are assessed routinely in Ecological Risk Assessment (ERA). Indirect effects include adverse effects on a species due to effects on other ecosystem components, such as a reduction in food abundance, a reduction in competition from another species, or a change in or loss of habitat. These indirect effects have not routinely been assessed, even though it is recognized that they may have as much, or more impact on ecological populations and communities as direct chemical toxicity. Not considering indirect effects can result in a significant source of uncertainty for the ERA. This is particularly important at larger, more complex sites (e.g., the Great Lakes ecosystem) where the cause of the impact may not be obvious. The traditional ERA process is well suited for adaptation to include the assessment of both predator/prey and habitat-related indirect effects. In addition, existing tools can be used or enhanced to address indirect effects. For example, mesocosm bioassays can be used instead of single species toxicity tests to address potential predatory/prey interactions. In addition, field survey techniques can be used to evaluate various habitat quality parameters. Addressing indirect effects requires good planning before the ERA begins, and development of comprehensive conceptual models of the potential interactions between ecosystem components. The data generated by the ERA then can be integrated into a weight-of-evidence analysis that uses causal analysis to identify the most likely source of impact(s).

Three proposed indicators of the risk of chemicals in fish and cost of consumption advisories. D.W. Smith. Conestoga Rovers and Associates, Exton, PA.

The science policy of the Great Lakes contains a disturbing dichotomy: potential risks from chemicals in fish are paramount to current policies, but appraisal of these risks is subject to very divergent and contentious estimates. The resulting dissensus frustrates rulemaking, inhibits compliance, and undermines the perceived legitimacy of regulatory agencies. Much of this dissensus is due to current indicators of risk/impact, which are poorly constructed and/or poorly understood and routinely misused. For example, the indicators are based on concentration (mg of chemical per kg of fish) instead of dose (mg of chemical ingested per person per day), the metric of toxicology. Current indicators also routinely confuse "worst-case" with likely scenarios. Three dose-based indicators of average risk/impact are proposed: (i) catch-weighted average concentrations; (ii) watershed per-capita dose of chemicals from recreational fish; and (iii) percent of watershed residents at risk/impacted by advisories. To illustrate their utility, they are applied to PCBs, the primary contaminant in Great Lakes fish, to contaminated Green Bay and Lake Michigan and relatively unimpacted Lake Erie. The indicators are calculated from available data, so implementation is inexpensive and easy. Moreover, compared to current indicators, the proposed indicators have greater sensitivity, transparency, and reliance on data and science, as opposed to policy and assumption. These characteristics should reduce discord. The third indicator, % of population affected, allows useful insight, e.g., it suggests that the at-risk population declines about twice as fast as fish concentrations.

Source water protection/Protection des sources d'eau

Session chair/Président: Timothy Fletcher

3D ecological modeling for assimilative capacity study of Lake Simcoe. Q. Lu[1], F. Duckett[1], N. Hutchinson[2], R. Baldwin[3] and R.B. Nairn[1]. [1]W.F. Baird & Associates, Oakville, ON; [2]Gartner Lee, Bracebridge, ON; and [3]Lake Simcoe Region Conservation Authority, Bracebridge, ON.

The Lake Simcoe Region Conservation Authority and the Province of Ontario are undertaking a series of studies to help them manage the pressures of population growth in the Lake Simcoe watershed. With rapid population growth and urban development comes additional land clearing, storm water runoff and the discharge of treated sewage, all of which are sources of increased phosphorus loading to Lake Simcoe. Depressed oxygen levels were linked to enrichment of the lake with the algal nutrient, phosphorus, the resultant stimulation of algal growth in the sunlit upper waters of the lake, and its subsequent senescence and settling into the hypolimnion where bacterial decomposition consumes oxygen from the stratified waters. This paper describes a 3D hydrodynamic and water quality model of Lake Simcoe developed using the Danish Hydraulics Institute MIKE3 model. The hydrodynamic module includes wind driven circulation, temperature variation, development of the thermocline and thermal stratification, and hydraulic forcing from inflowing tributaries. This is linked to the water quality module which allows the user to quantify the response of the lake to loadings of phosphorus, linking phosphorus loading to algal growth, the growth of aquatic plants and subsequent oxygen consumption. The model has been calibrated against measured temperature and water quality data at Ontario Ministry of Environment stations in the lake, and water intakes. The model is an important assessment tool for the management of Lake Simcoe and its watersheds to assess the impacts of the urban growth and land use change on the water quality in Lake Simcoe.

National Agri-environmental Standards Initiatives (NAESI) pesticide achievable performance standards development, method demonstration and verification project. P-Y Caux. Environment Canada, Environmental Conservation Service, Gatineau, QC.

As part of the Agricultural Policy Framework, Environment Canada is responsible for the National Agri-environmental Standards Initiative (NAESI), a program that will develop non-regulatory performance standards aimed at establishing the degree of desired environmental quality of air, water and soil in agricultural areas. This presentation will focus on pesticide Standards both Ideal and Achievable Performance Standards (IPS and APS, respectively) and on their verification in the field. To develop an APS, the farm system needs to go from a current state to a new desired state that will enable the reduction of pesticide run-off from fields. In the first year, we described this interval as a mathematical function, fRED, or the percent reduction in pesticide loss achievable using a optimal range of different Best Management Practices (BMPs). The concentration in the final receiving water body, predicted by a watershed model plus the BMPfRED, is an estimate of the level of environmental quality that is technically achievable through application of BMPs. This concentration can be adopted, unchanged, as the APS; or, it may be adjusted according to the socio-economics of BMP implementation to reflect the level of environmental quality that is likely to be achieved given the current technology and socio-economics. The APS verification project consists of parameterizing watersheds through modelling based on the hydrology, climatology, landscape characteristics and BMP usage. Choice models have been selected in order to provide watershed scale determinations of APS and being able to translate these to the farm scale through load allocations. The watershed models chosen were Basins, Swat and Gibsi. We are determining current baselines for watersheds by depicting probability density function that give the probability of pesticide loss with concentration.

A second, optimal predicted line is being generated through application of BMPs. This latter curve tends toward a more desirable state that would predict a lesser probability of a runoff for a pesticide. This year's efforts are focussed on the Chaudière River Qc. as a demonstration project. The pesticides under investigation are atrazine, nicosulfuron and dicamba. Monitoring data for these pesticides will serve to calibrate the models. The parameterization of several other river basins in Canada is being proposed based on the predominant commodity in that basin.

Water Quality Index (WQI): an alternate to the current Canadian Council of Ministers of the Environment (CCME) approach. N.K. Nagpal and L. Swain. British Columbia Ministry of Water, Land and Air Protection, Victoria, BC.

Water Quality Index has been used by various jurisdictions in Canada to express the environmental health of lakes and streams in their areas. The Canadian Council of Ministers of the Environment (CCME) designed approach is promoted for use to evaluate ecosystem health Canada-wide from a common perspective. However, the results obtained using the CCME approach depend upon a number of factors, require certain amount of data to be consistent, and do not always concur with the perception of the experts who are familiar with environmental health of waterbodies in their jurisdictions. We believe that the method proposed here is an improvement over the CCME approach. It is based on two basic assumptions: (i) a minor exceedance over the recommended water quality guidelines or the site-specific objectives does not mean that an ecosystem is impaired; and (ii) the ecosystem health degrades rapidly as the water quality guidelines or site-specific objectives approach the lowest observed effect levels. In general, the proposed approach yields results that suggest better water quality than those indicated by the CCME approach and are more in agreement with the experts' perception.

A methodology for derivation of ideal performance standards for pesticides in Canada. P.B. Jiapizian[1], P-Y. Caux[2], M. Bauder[1], D.R.J. Moore[3], R.S. Teed[3], R.L. Thompson[3], R. Breton[3] and R. Willis[3]. [1]Environment Canada, National Guidelines and Standards Office, Gatineau, QC; [2]Environment Canada, Environmental Conservation Service, Gatineau, QC; and [3]Cantox Environmental Inc., Ottawa, ON.

A goal of the Canadian Agricultural Policy Framework is to develop non-regulatory standards that specify desired environmental quality required of agriculture. As such, Ideal Performance Standards (IPS) are being developed for pesticides in Canada. IPSs are standards that protect biota in receiving environments affected by agricultural operations. The purpose of this presentation is to describe the methodology for deriving IPS for pesticides in surface water. In the methodology, decision criteria are used to select from six methods. The method selected depends on the available data. The methods include: (i) watershed-defined; (ii) site-specific species sensitivity distribution (SSD); (iii) generic SSD; (iv) full Canadian Water Quality Guideline (CWQG); (v) interim CWQG; or (vi) other approved benchmark. The watershed-defined method derives IPS for a specific watershed. Such IPS may be intended to protect locally-significant or sensitive species. The site-specific SSD and generic SSD methods are based on the SSD approach and estimate the concentration of a pesticide in water that will be protective of 95% of aquatic biota. The availability of site-specific data will determine the option selected. If data are insufficient for the SSD methods, the IPS can be an existing full or interim national Canadian Water Quality Guideline. The last method is to select a benchmark from another jurisdiction. These methods and a case study will be described and included in the presentation.

A source water protection plan protocol for First Nations. A. Klein and P.B. Jiapizian. Environment Canada, National Guidelines and Standards Office, Gatineau, QC.

In May 2003, Indian and Northern Affairs Canada, Health Canada and Environment Canada, jointly announced the seven-part First Nations Water Management Strategy, aimed at improving the safety of water supplies in First Nations communities south of 60° and in the Yukon. The strategy will be implemented over five years, and provides for the coherent and structured management of water quality on reserves, consistent with the multi-barrier (source to tap) approach. This includes protecting source water from pollution such as wastewater effluents, as well as providing effective drinking water treatment and distribution of drinking water. Public health and water quality experts around the world agree that the multi-barrier approach (MBA) should be followed to ensure that drinking water is safe. The MBA consists of the management of the drinking water source, appropriate water treatment and management, and well-maintained and safe water distribution systems. Source water protection is the first barrier in the approach and relies on measures which are generally preventive in nature. In some situations, as is the case with groundwater, it affords the only protection consumers have. This project represents the first federal guidance for the protection of the drinking water source in First Nations communities and other federal lands. The components of the Source Water Protection Plan Protocol include a source water assessment and the development of a source water protection plan. The source water assessment determines the level of risk to the water. A source water assessment must include the following 3 elements: (i) delineation of source water protection areas; (ii) identification of contaminants of concern through various inventories (contaminant inventories, land use inventories); and (iii) assessment of risk vulnerability and rank. As this guidance is geared specifically for First Nations, the incorporation of aboriginal traditional knowledge is specifically considered in the development of the protocol. The source water protection plan responds to the level of risk revealed by the source water assessment by addressing improvements that must be made in and around the water source to facilitate meeting the Canadian Drinking Water Guidelines at the tap of the consumer. The implementation component may also set targets for watershed improvement activities, while maintaining its focus on protection of the drinking water source.

Corridors and watersheds as management units/Corridors et bassins versants comme unités d'aménagement

Session chair/Président: Douglas Haffner

Distribution of heavy metals in sediments of the Huron corridor – local and corridor issues. E. Szalinska, K.G. Drouillard and G.D. Haffner. Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON.

The spatial distribution of 19 metals in sediments of the Huron corridor was established using data from a river-wide survey. The survey (2004) was based on a stratified random sampling design that encompassed four reaches of the corridor: the St. Clair River, St. Clair Delta, Lake St. Clair and the Detroit River. This investigation revealed that the metal contamination issue and pattern of its distribution are not uniform for the whole corridor. The St. Clair River, and subsequently the St. Clair Delta and Lake St. Clair, are still mainly affected by Hg contamination. Due to sediment remediation actions in the last years, the range of Hg concentrations decreased noticeably, but the same pattern of distribution has been remained from over 30 years. This pattern reflects mostly historical sources of Hg, however, elevated concentrations in sediments from the St. Clair River above the severe effect level indicate the activity of on-going sources of Hg. Active sources of Hg have been attributed as well to the Detroit River. This portion of the corridor has as well an essential significance for distribution such metals as Cr, Cu, Fe, Ni and Pb. In 33 to 75% of all sampling sites concentrations were in the range severe effect level and lowest effect level values which qualify these sites as

requiring a quantification of bioavailability of metals before developing remedial action plan priorities. A comparison between this investigation and a survey performed in 1999 indicates that the impact of metals carried from the Detroit Rivers into Lake Erie seems has not been reduced over the last 5 years.

Assessing zoobenthic community condition in the Lake Huron - Lake Erie corridor. J.J.H. Ciborowski[1], K.G. Drouillard[2], J. Zhang[1], E. Szalinska[2], A. Kirkpatrick[1] and G.D. Haffner[2]. [1]Department of Biological Sciences, University of Windsor, Windsor, ON; and [2]Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON.

Environmental assessments of aquatic habitats subject to multiple anthropogenic stressors are typically made by comparing observed community composition and relative abundance to that found in reference areas with comparable natural environmental attributes. This approach is limited when either suitable reference locations don't exist or when the boundary between reference and nonreference is difficult to ascertain. We compared 2004 zoobenthic community composition at 100 locations in the Detroit River, Lake St. Clair and St. Clair River chosen according to a stratified random design with data from earlier surveys. Distribution and abundance of indicator taxa representative of Detroit River depositional zones (*Hexagenia* mayflies and tubificid worms) suggested that fewer sites would be considered "equivalent to reference" and in 2004 relative to earlier surveys conducted since 1980. However, no more sites would necessarily be considered "degraded". Multivariate analysis delineated two biological assemblages of biota that we propose as "equivalent to reference" and "equivalent to degraded" for soft sediments of the Detroit River. A reciprocal-averaging approach, used to ordinate the relative composition of samples collected in 1998 and 2004 between these two compositional classes provides a method to spatially delineate the relative condition of benthic communities of depositional sediments between these two extremes. Preliminary evaluation suggests a relatively poor correspondence between their distribution and the spatial extent of sediment contamination with trace metals and hydrophobic organic contaminants.

GIS and numerical water and sediment transport models for application in watershed management systems. A. Brunton and T. Possley. W.F. Baird & Associates, Oakville, ON.

Quantitative analysis of water and sediment movement through watersheds is central to implementation of water quality and erosion control measures. Water and sediment flux pathways are tightly linked to contaminant transport, so understanding hillslope and channel sediment dynamics is vital to mitigation of catchment pollution. Computational tools to evaluate water flow and sediment transport have been developed as part of the Great Lakes Tributary Modeling Program. Integration of hydrologic, sediment yield, channel hydrodynamic and sediment transport models into GIS-based watershed management systems is still in its infancy, and models are often uncoupled, or loosely-coupled. This is problematic when appraising the potential impacts of scenarios such as the influence of land use or climate change on catchment sediment and nutrient delivery. Different types of modeling activities assist in Best Management Planning of watershed sediment issues. Numerical models of watershed hydrology and sediment delivery (SWAT; GSSHA), 2-D and 3-D river hydrodynamics (RMA2; EFDC), and channel sediment transport (SED2D) were constructed to develop an understanding of the hydrologic and geomorphic behavior of these systems, and to predict the effects of land use and riparian buffer modification. The models are linked through a common GIS environment, based around the ArcHydro data model, watershed spatial and temporal data layers, and a geodatabase of geomorphologic field survey data. Applications of these tools to water quality and sediment issues in several Great Lakes watersheds, along with the challenges of combining these diverse approaches and their implementation in best management practices are discussed.

Variability of PCB in water of the Detroit River - a long term mussel biomonitoring. K.G. Drouillard, A. Raeside, E. Szalinska, G.D. Haffner and T. Leadley. Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON.

Freshwater mussel (*Elliptio complanata*) biomonitors were deployed at six locations in the Detroit River during the open water season over the period of 1996 to 2003. This large temporal data set (n = 544 samples) was interpreted along with additional spatial biomonitoring and sediment quality surveys performed throughout the entire Detroit River during 1999 and 2002. Both mussel biomonitors and sediment surveys identified similar near-shore contaminated regions within the Detroit River indicating that on-going loadings of PCBs to water continue to occur within this system. A toxicokinetic model was used to interpret time-accumulated residues measured in mussel tissues at individual sites and years. Although lipid normalization removed a portion of the site- and time-specific individual variation in mussel exposures, steady state correction of time-accumulated mussel residues failed to remove within site and within-year variation for the majority of cases. There were only a few exceptions (sites and years) where non-steady state, lipid-normalized contaminant residues in mussels followed the expected uptake trajectory predicted by the bioaccumulation model under a condition of constant water concentrations. These observations implicate considerable variation in PCB water concentrations in the Detroit River during the open water season. Passive monitoring methods such as those established by biomonitors or continuous monitoring strategies as afforded by real-time instrumentation should be used in conjunction with traditional grab sampling or high-volume water sampling approaches to adequately characterize PCB and organochlorine loads to and from the system.

Long-term trends of volatile organic compounds in the St. Clair River. S. Munro[1] and E.J. Kuley[2]. [1]Sarnia-Lambton Environmental Association, Sarnia, ON; and [2]ORTECH Environmental, Sarnia, ON.

The Sarnia-Lambton Environmental Association, a non-profit, industry-based environmental co-operative located in the Sarnia area, has maintained a program of fully automated hourly sampling of the St. Clair River for specific volatile organic compounds (VOCs) since 1986. Design expectations of the system and details of the original installation, along with evolutionary improvements and recent progression to a second generation system will be reviewed. With method detection limits in the 0.05 to 0.16 $\mu g \cdot L^{-1}$ range for most of the 20 VOCs routinely analyzed, the current system has provided a unique opportunity to track improvements in water quality at extremely low levels. The system reports and archives data hourly, allowing Association members to access "real-time" data in the event of an emergency, sharing the data with regulators to assist in managing the event. The system also automatically alarms at predetermined levels, to allow remote checks of operating status, and to provide a secondary alert regarding possible spills to the river. The sampling system also provides automated grab samples, retained on a repeating 3 h cycle, to allow laboratory confirmation of results by GC/MS, or extension to additional analytes as necessary. Long-term trends in St. Clair River water quality will be reviewed, along with the instrument's response to a typical spill event.

Environmental indicators of Great Lakes coastal margins: measuring and linking physical, chemical and biological integrity. J.J.H. Ciborowski[1], G.J. Niemi[2], N.P. Danz[2], R.R. Regal[2], L.B. Johnson[2], V.J. Brady[2], J.R. Kelly[3] and R.W. Howe[4]. [1]Department of Biological Sciences, University of Windsor, Windsor, ON; [2]Center for Water and the Environment, Natural Resources Research Institute, University of Minnesota Duluth, Duluth, MN; [3]U.S. Environmental Protection Agency, Mid-Continent Ecology Division, Duluth, MN; and [4]Department of Natural and Applied Science, University of Wisconsin Green Bay, Green Bay, WI.

The condition of an ecosystem reflects the complex interactions among the biota and their interaction with the physical and chemical characteristics of their landscape. The strongest linkages occur between the biota of connecting channels and coastal margins and their contributing watersheds. Environmental indicators are surrogates that describe this condition. The State of the Lakes Environmental Conference (SOLEC) develops suites of indicators that inform about the integrity of the Great Lakes as a whole. Local Remedial Action Plan teams adapt indicators most relevant to their Areas of Concern. Nevertheless, the best indicators are easily expressed and understood, quantitative, bounded (have defined maximum and minimum values), and meaningful. The advent of agency-sponsored data-rich web sites has made databases of physical and chemical measurements of environmental condition easily accessible. But their meaning may be unclear because many stressors co-occur in degraded areas. Multivariate methods and GIS representation permit one to summarize, simplify and map huge amounts of data. Measures such as the Great Lakes Environmental Indicators (GLEI) index of vulnerability summarize how different classes of environmental pressures vary at the Great Lake and connecting channel margins among 2nd-order watersheds, and indicate which stress may be greatest in any locale. Biological measurements are needed to demonstrate whether stress actually affects biological integrity. Calibrating how biota change in relation to these pressures helps identify the point at which physical and chemical conditions cause beneficial use impairments or what criteria should be met to warrant delisting an Area of Concern. Across the U.S. Great Lakes, agricultural activity most frequently exerts greatest pressure followed by pressures associated with population density. Together, simple indicators of the physical, chemical and biological condition of an area can summarize overall condition and suggest most appropriate remediation strategies. However, indicator "averages" tend to inaccurately portray a sense that an ecosystem is in some intermediate state between excellent and poor. Perhaps composite score scales should represent degrees of certainty of the state of environmental condition rather than its nearness to a desired state.

Introducing a method to derive achievable performance standards at the sub-watershed level for pesticides. S. Goldacker[1], J. Archbold[1], J. Shapiro[1], P.B. Jiapizian[2], P-Y. Caux[2], R.S. Teed[3] and D.R.J. Moore[3]. [1]Cantox Environmental, Mississauga, ON; [2]Environment Canada, National Guidelines and Standards Office, Gatineau, QC; and [3]Cantox Environmental, Ottawa, ON.

A methodology to derive Achievable Performance Standards (APS) at the sub-watershed level for in-use agricultural pesticides was developed. An APS is a maximum pesticide concentration in surface water, set at the level that can be technically achieved through the application of Best Management Practices (BMPs) to agricultural fields in a sub-watershed. The steps of the method are to: (i) identify the sub-watershed and pesticide for which the APS is to be developed; (ii) parameterize the watershed model with sub-watershed and pesticide specific data; (iii) identify BMP efficacy data for similar pesticides and site conditions; (iv) calculate a BMP reduction function (fRED) from the BMP efficacy data using a statistical treatment that is appropriate for the level of specificity of the efficacy data to the situation; and (v) apply fRED to the pesticide concentration predicted for the sub-watershed under baseline conditions to calculate the achievable pesticide concentration. This method relies on the development of a Canadian watershed model that can integrate: (i) fate and transport of pesticides through the hydrological system; and (ii) impacts of baseline agricultural practices on pesticide loss from fields to calculate a pesticide concentration in the receiving water body of a sub-watershed. Significant development of BMP efficacy data and detailed GIS characterization of watersheds will be needed before the method can be applied broadly in Canada. The advantages and limitations of the method are discussed using results of a case study based on a hypothetical situation in a U.S. watershed.

Thermal impact assessment of the St. Clair River. T.S. Moran[1], J. Houtby[1], and S. Munro[2]. [1]Pollutech EnviroQuatics Ltd., Point Edward, ON; and [2]Sarnia-Lambton Environmental Association, Sarnia, ON.

This exploratory study was commissioned to improve understanding of potential ecological impacts associated with thermal discharges to the St. Clair River ecosystem. The study was designed to establish a baseline from which to track temporal trends. Initial results will identify existing conditions and seasonal trends in the thermal profile of the river; establish relationships between point source heat load and seasonal temperature fluctuations in the receiving environment; and, assist in developing a study design for assessing ecological impacts of thermal discharges. Water temperatures are being monitored at three depths *in situ*, using continuous temperature data loggers at 16 locations along the length of the St. Clair River, primarily in the vicinity of point source discharges to the St. Clair River and its tributaries. The first phase of this study (May through December 2005) initiates a long-term monitoring program to assess both short and long-term impacts of thermal discharges. The data set generated by this study will be useful to site managers in making sound environmental decisions; in working with regulators; in helping to demonstrate compliance with site-specific regulatory requirements; and, in designing a study to address the risks associated with significant thermal discharges. Temperature profiles along the river at three depths will be presented for the spring / summer 2005 seasons.

Areas of Concern – environmental health/Secteurs préoccupants - santé environnementale

Session chairs/Présidents: James Sherry, Scott Brown and/et Mark McMaster

Status of beneficial use restoration for fish tumors and deformities at Great Lakes Areas of Concerns. S.B. Smith[1] and P.C. Baumann[2]. [1]U.S. Geological Survey, Contaminants Biology Program, Reston, VA; and [2]U.S. Geological Survey, Leetown Science Center, Columbus, OH.

Contaminated sediments have been the focus for observing biological impacts at the Great Lakes Areas of Concern (AOCs). These impacts include the health and well being of various fish populations. An elevated prevalence of liver cancer and external anomalies in fish populations from Great Lakes tributaries caused tumors and other deformities to be among the Beneficial Use Impairments in Annex II of the 1987 Protocol Amending the *Great Lakes Water Quality Agreement* of 1978. This beneficial use is considered impaired: "When the incidence rates of fish tumors or other deformities exceed rates at unimpacted control sites ...". External anomalies and cancerous liver tumors are important considerations for evaluating the restoration of beneficial use. External anomalies prevalence above 15% is considered impaired for brown bullhead (*Ameiurus nebulosus*) and above 10 to 20% for white suckers (*Catostomus commersoni*), depending on southern vs northern Great Lakes. The most prevalent external anomalies in Lake Erie brown bullhead were abnormal barbels, raised growths on the skin and lips, focal discoloration (melanoma) and blindness. The fish tumor beneficial use impairment occurred at 16 of the 41 AOCs. The systems having the lowest incidence (reference conditions) of confirmed liver tumors approach a 0.5% prevalence in age 3 fish and a 2% prevalence for ages 4 and 5 combined. In order to standardize sample collections, monitoring should be done in spring and fish should be compared by single (age 3) and double (ages 4 & 5 and 6 &7) age groups. A Great Lakes wide effort to compare AOCs with reference conditions and to determine acceptable conditions for liver cancer and external anomalies in nearshore fish populations in each individual Great Lake needs to be conducted. Monitoring, especially at reference

sites, using internationally consistent methods is imperative to determine restoration of beneficial use at Great Lakes AOCs.

Fish tumors and other deformities - establishing diagnostic criteria. V.S. Blazer[1], L. Iwanowicz[1], E. Obert[2] and J. Grazio[3]. [1]U.S. Geological Survey, National Fish Health Research Laboratory, Kearneyville, WV; [2]Pennsylvania State University, Pennsylvania Sea Grant, Eire, PA; and [3]Pennsylvania Department of Environmental Protection, Meadville, PA.

One of the beneficial use impairments (BUI) at Areas of Concern (AOC) in the Great Lakes is listed as "fish tumors and other deformities" and defined as occurring when "the incidence rate of fish tumors and other deformities exceeds rates at unimpacted or control sites or when survey data confirm the presence of neoplastic or preneoplastic liver tumors in bullhead or suckers". Currently, 16 of the 41 AOCs have the presence of tumors or other deformities listed as one of their beneficial use impairments. A key indicator species for AOCs, as designated by International Joint Commission, is the brown bullhead (*Ameiurus nebulosus*), a bottom-dwelling fish with a small home range, known to take up contaminants from food and sediments. Both liver and skin neoplasms of bullheads have been associated with polyaromatic hydrocarbons and other contaminant exposure. Recent studies at two AOC, the Ashtabula River and Presque Isle Bay (recently listed as an Area of Recovery) has raised some issues concerning this BUI. These include the standardization of the criteria being used to evaluate the impairment and setting realistic delisting targets. Criteria need to be established for both sampling and monitoring this impairment so that data from different years, reference sites and Areas of Concern can be compared. It is evident from the literature that different pathologist/investigators have use different criteria or definitions for neoplastic and preneoplastic liver lesions. In addition, although neoplasia must be diagnosed microscopically, incidence in some studies is based on gross observations (particularly for skin lesions) while in others is based on histopathology. We will present an attempt to establish diagnostic criteria for proliferative liver and skin lesions of bullheads. Data will also be presented from a number of reference sites sampled over a three year period for the Presque Isle Bay study and one reference site sampled seasonally during the Ashtabula study. Factors such as age, seasonal differences, number of liver pieces sampled, and combination of reference site data (for increased sample sizes) in order to determine "background" incidence of various lesions, will be discussed.

Assessment of wild fish from some Canadian Areas of Concern for fish health and reproductive alterations. G.R. Tetreault, M.E. McMaster, C. Boyko, L.M. Hewitt, T. Morgan, J.P. Sherry and S.B. Brown. Environment Canada, National Water Research Institute, Burlington, ON.

Environment Canada has undertaken studies in Canadian Areas of Concern (AOCs) to determine the current state of fish and wildlife health. Phase One (2001 to 2005) is focusing on conditions in AOCs of the lower Great Lakes. The studies presented here will focus on AOCs in the Bay of Quinte, the St. Lawrence River at Cornwall, and the Niagara River, Ontario. Two different fish species were collected from exposed and reference sites and examined for reproductive health. As part of this study our laboratory measured circulating levels of reproductive sex steroids, and the capacity of gonadal tissue to produce these steroids using an *in vitro* incubation assay. These reproductive endpoints were compared to other indicators of overall fish health and the presence of ligands for fish sex steroid receptors recovered from semi-permeable membrane devices (SPMDs) deployed for 21 d where wild fish were collected. Yellow perch (*Perca flavescens*) collected within the Bay of Quinte were impacted relative to a reference population with decreased condition, increased liver size, decreases in gonadal development, complimented with decreases in circulating steroid levels and reduced ability to produce sex steroids. Brown bullhead (*Ameiurus nebulosus*) had similar results although not as

severe with increased liver size and decreased circulating sex steroid levels. Ligands for the goldfish androgen receptor were detected in SPMD extracts at the mouth of the Trenton River, Trenton. All other SPMDs showed no accumulation of hormonally active compounds. Fish collections at Cornwall again demonstrate health impairments with decreased energy reserves and lower capacity to produce sex steroids by brown bullhead. Cornwall yellow perch had increases in liver size downstream of the dam, and altered steroid production capacity. Studies on the Niagara River revealed alterations in liver size and circulating T and 11-KT in male fish collected at the far-field Queenston site. This study is ongoing and its overall goal is to reassess all of Canada's AOCs for evidence of endocrine disruption.

Are feral fish in Great Lakes' Areas of Concern exposed to environmental estrogens? J.P. Sherry, C. Tinson, T. Hooey, M.E. McMaster, L.M. Hewitt and S.B. Brown. Environment Canada, National Water Research Institute, Burlington, ON.

As part of a broader assessment of wildlife health in Areas of Concern (AOC) on the Canadian side of the Great Lakes we have examined feral fish for evidence of exposure to both environmental estrogens (EEs) and anti-estrogens. For the purposes of this study we divided each AOC into upstream, impact zone, and downstream sites. At each site we sought to capture 20 adult male and female specimens of a bottom dwelling and a pelagic fish species. We were able to meet our quota at most, but not all, AOCs. After a general health assessment a blood sample was taken from each fish. The plasma was separated and stored in liquid nitrogen prior to analysis. Vitellogenin (Vg) was measured by means of a sensitive electrophoresis technique which shall be described. Presence of Vg in the plasma of male fish indicates exposure to EEs. A decreased level of plasma Vg in female fish from the impact zone compared with levels in females from the reference location indicates that the fish may have been exposed to anti-estrogens. We present the results for the Wheatley Harbour, Detroit River, and St. Clair River AOCs. The data suggest that feral fish at the St. Clair and Detroit River AOCs have may been exposed to EEs. Vg was measured in most male goldfish (*Carassius auratus*) taken from Wheatley Harbour and nearby Hillman Marsh, which served as a reference location. Lower concentrations of Vg ($p < 0.01$) were measured in female goldfish from Wheatley Harbour than from Hillman Marsh. Further research will be required to confirm the observed effects and to determine possible causes.

Assessment of thyroid status in fish from Great Lake Areas of Concern. S.B. Brown[1], R.E. Evans[2], M. Villella[1], M.E. McMaster[1] and J.P. Sherry[1]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

Problems with the thyroid gland, mainly observed as large goiters and thyroid microfollicular hyperplasia, have been observed in Great Lakes salmonids for more than 30 years. We employed a recommended minimum suite of assays necessary to detect perturbations in the thyroid axis of fishes from Areas of Concern (AOCs) in the lower Great Lakes. The assays are capable of detecting changes in thyroidal production of T4 and in the peripheral production of T3. We found evidence that fish at near-field locations at the AOCs are responding to the presence of factors that influence peripheral metabolism of thyroid hormones. However, the effects fall within the compensatory scope of the thyroidal system because there is little evidence of changes in thyroid gland histology.

Beyond the toxicity test: St. Clair River sediment remediation of the nearshore Dow outfall area. T.S. Moran[1], N. Orr[1], J. Houtby[1] and C. Creber[2]. [1]Pollutech Enviroquatics Ltd., Point Edward, ON; and [2]Dow Chemical Company of Canada Inc., Sarnia, ON.

Dow Chemical Canada Inc. (Dow) has implemented numerous measures to mitigate any potential

impact to the St. Clair River associated with the historical operations on the Sarnia Site. These measures were focussed initially on source elimination; removal of chemicals in water discharged to the river, separation of sewers, reduction of discharge point/outfall reduction; collection and monitoring of all stormwater; and, more recently, involved the removal of the contaminated sediments in the near shore area along their river frontage (i.e., Dow Outfall Area). The contaminants of concern in these sediments were mercury, semi-volatile organic compounds including hexachlorobenzene, hexachlorobutadiene, octachlorostyrene, and volatile organic compounds including 1,2-dichloroethane, carbon tetrachloride, and tetrachloroethylene. A risk management approach was employed to evaluate potential remedial alternatives (goals/effectiveness, implimentability, and cost) and included extensive regulatory and public consultation. Dow selected a combination of a hydraulic sediment removal technology and mechanical excavation, with a combination of landfill disposal and treatment of the sediments once removed. The hydraulic sediment removal process removed sediment and transferred it to an onshore treatment facility, where the water was separated from the sediment and treated before discharge back to the St. Clair River. Solids were treated such that they could be disposed of as either non-hazardous waste or remain on site to be treated using bioremediation technology. A three-phased, three-year approach was selected. The first phase allowed for the evaluation of the effectiveness of the sediment removal technology, water treatment processes, environmental control, and monitoring procedures. Subsequent years then allowed for management of sediments based upon different zones of contamination requiring different management and treatment strategies. Dow's sediment remediation project initiated in 2002 was completed in 2005 with the application of washed river stone to act as habitat enhancement. This presentation will provide an overview of the entire project with key messages addressing the need for community communications prior to and during the project, operational risk management, environmental diligence to minimize downstream impacts and the benefits and limitations of such an operation.

Assessing neuroprotective P-glycoprotein activity in killifish (*Fundulus heteroclitus*) exposed to aquaculture pesticide using behavioural profiles. S.M. Bard[1], K. Menashe[2], P. Santarelli[1] and S. Gadbois[3]. [1]Environmental Programmes, Dalhousie University, Halifax, NS; [2]Department of Biology, Dalhousie University, Halifax, NS; and [3]Department of Psychology and Neuroscience, Dalhousie University, Halifax, NS.

A behavioural toxicological assay was developed to determine the neuropathological effect of the aquaculture pesticide and anti-parasitic drug ivermectin in killifish (*Fundulus heteroclitus*). We developed this assay to examine the neuroprotective role of P-glycoproteins (P-gps), multixenobiotic resistance transporters, in the blood-brain barrier in fish. P- glycoproteins act as cellular efflux pumps to prevent substrates from accumulating in the brain, including environmental contaminants such as ivermectin. P- glycoprotein function and thus blood-brain barrier integrity can be compromised by chemosensitizers which inhibit transport activity. Fish treated with ivermectin and the P-glycoprotein inhibitor Cyclosporin A were significantly more sensitive and succumbed more rapidly to melanosis, tilting, lethargy, slowing of pectoral fin movement and loss of haptic reactivity compared to fish treated with ivermectin only. P-glycoprotein inhibition is associated with significantly earlier onset and increased mortality in ivermectin-exposed fish. Our results suggest that P-gps confer resistance against ivermectin- induced behavioural neuropathology and mortality in fish. This assay provides us with a non-invasive tool to study P-gps function in the blood-brain barrier and evaluate the behavioural effects of potential environmental neurotoxins.

PCB contamination in Lyons Creek: implementation of a decision-making framework for sediment. R. Fletcher[1] and D. Milani[2]. [1]Ontario Ministry of the Environment, Environmental

Monitoring and Reporting Branch, Etobicoke, ON; and [2]Environment Canada, National Water Research Institute, Burlington, ON.

The International Joint Commission has identified the Niagara River as an Area of Concern. Lyons Creek, a tributary of the Niagara River, has been identified as a potential source of PCBs to the river. In 2002 and 2003, the Ministry of the Environment and Environment Canada undertook an extensive study of the eastern portion of Lyons Creek to determine the extent of PCB contamination in the creek. Data from this study were used to assess ecological condition of the creek using a risk-based decision-making framework for the management of contaminated sediment, recently developed by the *Canada-Ontario Agreement* Sediment Task Group. This framework provides a step by step approach to assess contaminated sediments, and is based on the ecological risk assessment principles. The overall assessment of each Lyons Creek site was achieved by integrating the information obtained both within and among four lines of evidence: sediment chemistry, benthic invertebrate community structure, sediment toxicity and the biomagnification potential of PCBs. Collections of resident sport fish in the creek provided ground-truthing of the model, as well as demonstrating bioaccumulation of PCBs in higher trophic level organisms. The placement of mussels at select locations in the creek provided an indication of the bioavailability of contaminants from the water column, as well as providing an indication of availability in areas where resident biota were unavailable.

Antibiotic resistance identified in water sources in the Durham region, Ontario. S.E. Keeling[1], L.J. Praetorius[1], C.L. Burton[2] and D.A. Holdway[1]. [1]Faculty of Science, University of Ontario Institute of Technology, Oshawa, ON; and [2]School of Applied Science, Durham College, Oshawa, ON.

Bacteria such as *Escherichia coli* are used to indicate the level of fecal pollution in various water sources. As *E. coli* are readily found in both animals and humans, it is difficult to identify the specific source of the pollution, however their presence indicates the likely presence of other pathogens that may be detrimental to human health. The identification of antibiotic resistance patterns and study of clonal line similarities can provide valuable information on the molecular ecology of bacteria associated with fecal pollution. Two study areas in the Durham region of Ontario (one recreational and one wildlife area) each with three sampling sites were investigated to identify the different antibiotic resistant biotypes and clonal lines present in a subset of randomly selected fecal coliforms. Resistance to ampicillin, streptomycin and kanamycin was most common amongst sites, however, resistance was evident for all antibiotics tested (ampicillin, ciprofloxacin, chloramphenicol, gentamicin, florfenicol, kanamycin, nalidixic acid, streptomycin, tetracycline). To date 66 different biotypes have been identified. Preliminary results of this study have shown that biotype patterns can vary substantially over temporal and spatial distances.

Environment Canada's screening assessment of existing substances: An update on the pilot project. W.M. Windle and R. Chénier. Environment Canada, Existing Substances Branch, Gatineau, QC.

Under the *Canadian Environmental Protection Act*, 1999 (CEPA 1999), all substances found to be persistent and/or bioaccumulative and inherently toxic during the "categorization" of Canada's Domestic Substances List (DSL) must undergo a screening assessment to determine whether the substance is "toxic" as defined under the Act. The DSL contains about 23,000 substances and numerous screening assessments are expected to be prepared. Therefore, a pilot project involving 123 substances was initiated to develop science, policy, and administrative approaches and methods for assessing substances identified through categorization. This presentation will provide an overview and update on the pilot project screening assessments.

Soil and groundwater remediation through the program of energy research and development at Environment Canada. P.C.B. Bacchus. Environment Canada, Environmental Conservation Service, Gatineau, QC.

Environment Canada (EC) has been an active participant in the federal Program of Energy Research and Development (PERD) since 1977. Together with other federal departments, universities, and industry sectors, EC through PERD has contributed to finding sustainable energy development solutions for the benefit of Canadians. This participation has led to the advancement and sharing of knowledge in technologies, and will also contribute to ensuring sustainable development in northern and frontier regions. Science and technology efforts under PERD support decision-making, policy development, and regulatory activities; contribute to the development of national standards and guidelines; support public safety; and environmental needs. Through PERD, EC has developed a high level of expertise which has led to the advancement of knowledge in a number of priority areas allowing for timely advice on emerging and existing issues. One area of expertise is to address groundwater and soil remediation issues. This research and development (R&D) is conducted in the context of activities related to the oil and gas industry. Contamination from the oil and gas industry of groundwater and soil is a serious issue, since these are fundamental factors controlling the health of ecosystems and economic viability of the land. The R&D presented outlines the activities occurring within this area and how the Program will advance the development of better generic remediation technologies/approaches for use by industries as well as the development of key guidelines, methods and protocols to be used by regulators.

Microcystin in sport and pan fish from Lake Erie. R. Schuster[1], J. Telecky[2] and P.F. Dehn[1]. [1]Department of Biology, Canisius College, Buffalo, NY; and [2]New Your State Department of Environmental Conservation, Lake Erie Fisheries Unit, Dunkirk, NY.

Cyanobacterial blooms containing *Microcystis aeruginosa*, which produces a potent hepatotoxin, have been reported in the western portion of Lake Erie since 1995, and public health advisories have been issued since 1998. Recent studies in South America indicate the presence of microcystin toxin in fish muscle following blooms, which pose a potential health risk to humans and other organisms who consume contaminated tissues. The purpose of this study was to determine whether or not this toxin could be found in muscle tissue of common sport and pan fish from the eastern end of Lake Erie. Eight yellow perch (*Perca flavescens*), 9 smallmouth bass (*Micropterus dolomieui*), 6 lake trout (*Salvelinus namaycush*), and 6 adult and 9 yearling walleye (*Sander vitreus*) were collected, fillets were homogenized, extracted in methanol, and analyzed for microcystin using an enzyme-linked immunosorbent assay. Microcystin ($\mu g \cdot kg^{-1}$ wet tissue) was present in all species (yellow perch 17.3 + 10.1, smallmouth bass 19.1 ± 14.8, lake trout 12.5 ± 2.7, walleye yearling 16.6 ± 8.4, and adult walleye 12.6 ± 7.4). Based on U.S. EPA's meal size of 227 g, ll reported levels exceed WHO's guidelines for a 90 kg individual consuming four fish meals per month, which have been shown to be realistic consumption levels for sports fishers from Lake Erie; suggesting sports fishers are at risk.

Occurrence of endocrine disrupting alkyphenols in Cootes Paradise marsh, Ontario. T. Mayer[1], D.T. Bennie[1], F. Rosa[1], G. Rekas[2], V. Palabrica[1] and J. Schachtschneider[1]. [1]Environment Canada, National Water Research Institute, Burlington, ON; and [2]Department of Biology, MacMaster University, Hamilton, ON.

Cootes Paradise Nature Sanctuary is an important spawning ground for fish and a crucial habitat for other fauna. Occurrence and fate of alkylphenols (APs), known endocrine disruptors in this Great Lakes coastal wetland was investigated. Of concern are nonylphenol ethoxylates (NPEs) and their degradation products such as nonylphenol (NP). Although low concentrations of alkylphenols were

detected in open water of the marsh, high levels of the NPEs (4 to 33 $mg \cdot kg^{-1}$ of NP2E and as much as 91.7 $mg \cdot kg^{-1}$ of NP3-17E) were measured in areas near point sources. The distribution of APs in sediments varied, with the highest concentrations near the sewage treatment plants (STP) and CSO outfalls, where the 4-NP concentrations in surficial sediments varied between 1.5 and 4.6 $mg \cdot kg^{-1}$. Highest 4-NP concentrations (8.8 $mg \cdot kg^{-1}$) were found 25 cm below the sediment surface at the mouth of Chedoke Creek, which conveys combined sewer overflow outfall from Hamilton. This peak corresponds to 1954, when Hurricane Hazel struck southern Ontario and dumped more than 200 mm of rain on Cootes Paradise watershed. Large volume of runoff, transporting various contaminants bypassed the Hamilton STP and flowed via Chedoke Creek directly into Cootes Paradise. Since transfer of APs through the food chain is of concern, we compared their concentrations in invertebrates from clean and contaminated sites. The results reveal accumulation of these contaminants in the invertebrate tissue, particularly the highly lipophilic 4-NP, whose concentrations ranged from 1.9 to 6.3 $mg \cdot kg^{-1}$. This is the first study to evaluate AP concentrations in tissue of benthic invertebrates.

Biological assessment of PCB contamination in Lyons Creek (Niagara River Area of Concern) Welland, Ontario. D. Milani[1], R. Fletcher[2] and L. Grapentine[1]. [1]Environment Canada, National Water Research Institute, Burlington, ON; and [2]Ontario Ministry of the Environment, Environmental Monitoring and Reporting Branch, Etobicoke, ON.

As part of a Remedial Action Plan developed for the Niagara River, tributaries of the river, including Lyons Creek, were recognized as part of the Area of Concern. Increased population, industry and agriculture in the Niagara River watershed have subjected the river to increased levels of pollutants including polychlorinated biphenyls (PCBs). To assess the biological impacts of sediment PCB contamination, Lyons Creek (East) and four neighbouring creeks (reference) were sampled. Physico-chemical properties of the sediment were considered, as well as resident benthic invertebrate tissue, benthic community structure, sediment toxicity, and forage fish, sport fish and mussel tissue. The upper area of Lyons Creek (from the Welland canal to Highway 140) has the highest levels of total PCBs (and metals) in the sediment (PCB range 0.02 to 19 $mg \cdot kg^{-1}$). These concentrations are up to 270 times higher than the sediment quality guidelines Lowest Effect Level (LEL) and higher than reference creek concentrations (PCB range 0.002 to 0.016 $mg \cdot kg^{-1}$). Concentrations in resident benthic invertebrates in the same area (PCB range 0.02 to 53 $mg \cdot kg^{-1}$) are also elevated above reference concentrations (PCB range 0.05 to 0.4 $mg \cdot kg^{-1}$), and acute toxicity is evident at three sites. Benthic communities are generally similar to reference with one site having a depauperate community compared to reference. Based on resident benthos PCB and biomagnification factors derived from the literature, PCBs were predicted to bioaccumulate in higher trophic level receptors (e.g., fish) to concentrations that are not protective of adverse effects at up to 11 sites. Forage and sport fish PCB are above guidelines in some cases and are highest in fish collected at Highway 140.

Environmental effects monitoring/Surveillance des effets environnementaux

Session Chair/Président: Nardia Ali

Developing reference benchmarks for the northern Ontario biomonitoring network. M.F. Bowman[1], C.I. Brereton[2], C. Sarrazin-Delay[3], B. Keller[4] and K.M. Somers[5]. [1]Department of Zoology, University of Toronto, Toronto, ON; [2]INCO, Environmental Health and Safety, Copper Cliff, ON; [3]Department of Biology, Laurentian University, Sudbury, ON; [4]Ontario Ministry of the Environment, Environmental Monitoring and Reporting Branch, Sudbury, ON; and [5]Ontario Ministry of the

Environment, Environmental Monitoring and Reporting Branch, Dorset, ON.

The northern Ontario benthic invertebrate biomonitoring network was designed to assist the metal mining industry in locating suitable reference sites to meet the Environmental Effects Monitoring requirements of the federal *Metal Mining Effluent Regulation.* The premise underlying this Reference Condition Approach is that relatively homogenous biological communities are expected to occur in areas with distinct sets of habitat characteristics. There are a number of traditional and novel methods that can be used to ensure a test community is compared to the appropriate set of reference communities. Due to drawbacks such as high misclassification rates, we investigated alternatives to these traditional approaches. For example, the nearest-neighbour approach does not involve arbitrary groupings and may be more appropriate for communities spanning gradients. We conclude with a list of pros and cons of traditional and novel methods for matching test sites with appropriate reference sites.

Investigation of cause for metal mining environmental effects monitoring (EEM): complexities and considerations. M.G. Dubé[1], L.P. Weber[1], C.J. Rickwood[1], K. Driedger[1], C.I. Brereton[2], D.M. Janz[3] and D.L. MacLatchy[4]. [1]Environment Canada, National Water Research Institute, Saskatoon, SK; [2]INCO Ltd., Safety, Health and Environment, Copper Cliff, ON; [3]Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, SK; and [4]Department of Biology, University of New Brunswick, St. John, NB.

Investigation of cause is considered after an effluent effect is measured, confirmed, and its spatial extent and magnitude quantified. Studies at INCO Limited in Sudbury, Ontario have included artificial stream bioassays, field studies in Junction Creek, laboratory investigations on juvenile fish bioenergetics, and comparison of waterborne and foodborne pathways of exposure to fathead minnow (*Pimephales promelas*) in a short-term reproductive bioassay. Artificial stream studies in 2001 and 2002 showed the effects of 45% Copper Cliff effluent were decreased adult survival and some effects on reproduction (decreased gonad size and reproductive hormones). Bioassays with *Chironomus tentans* in 2002 and 2003 showed this same effluent decreased larval survival, and cumulative egg production and emergence in adults. Field studies in 2004, showed increased metal body burdens and liver weight in male fathead minnow, and decreased egg size in fathead minnow and creek chub (*Semotilus atromaculatus*) downstream of the discharge. In 2004, exposure of fathead minnow to this effluent in a reproductive test showed decreased spawning events and egg production. Foodborne exposure of adults significantly affected larval survival where as waterborne exposure did not. These studies indicate that 45% Copper Cliff effluent, as tested, affected the measured species. Characterization of the effluent show a complex suite of potential causative agents. Development of IOC guidance requires consideration of the potential for realistic mitigation in the context of best treatment practice, extensive historical effects, and consideration of non-point sources.

Aquatic oligochaete, *Lumbriculus variegatus*, reveals gradients of pesticide exposure in the water column and in sediment. A. Alexander[1], J.M. Culp[2], D. Baird[3] and P.M. Williams[4]. [1]Department of Biology, University of New Brunswick, Fredericton, NB; [2]Environment Canada, National Water Research Institute, Fredericton, NB; [3]Environment Canada, National Water Research Institute, Wolfville, NS; and [4]Aquaculture Institute, University of Sterling, Stirling, United Kingdom.

Agricultural pesticides cause sublethal responses in aquatic invertebrate communities. *In-situ* bioassays were conducted in two pesticide affected streams by exposing the sediment dwelling aquatic oligochaete, *Lumbriculus variegatus*, to blank and natural sediments in nested pairs for 5 days. This design enables the study of the interaction of waterborne and sediment sources of contamination in an ecological context. Six riverine sample sites were examined in an agricultural area of northwestern

New Brunswick with five replicate pairs containing either blank or natural (site specific) sediments. Sublethal and lethal endpoints will be examined and compared to laboratory studies. The sublethal endpoint of egestion rate (mg^{-1} $worm^{-1}$ $hour^{-1}$) was measured post-exposure with significant differences found between exposure sites as well as between blank and natural sediments. Oligochaete *in-situ* bioassays are a useful biomonitoring tool and the use of blank sediments helps to determine sources of toxicity.

Use of *in situ* hatchboxes containing cutthroat trout (*Salmo clarki*) as a tool for environmental effects monitoring (EEM). B. Chambers[1], J.R. Elphick[2], H.C. Bailey[3] and G.C. Dirom[4]. [1]Breakwaters Resources, Ltd, Campbell River, BC; [2]Nautilus Environmental, Victoria, BC; [3]Nautilus Environmental, San Diego, CA; and [4]Natural Resources Canada, Environmental Assessment and Regulatory Division, Vancouver, BC.

Interpreting the results of fish population monitoring programs can be problematic due to the inability to control for the movements of organisms in the aquatic receiving environment and differences in habitat between reference and exposure areas, particularly in cases where the fish population and receiving environment is limited. Thus, the use of *in situ* bioassay studies may provide an opportunity to quantitatively evaluate the potential effects of a discharge (e.g., effluent) on key life-stages of salmonids under the conditions found in the actual receiving environment. This presentation describes the approach used at the Myra Falls Operations mine to evaluate the potential for effects on fish populations in the receiving environment from mine-related discharges. Eyed-stage cutthroat trout *(Salmo clarki)* embryos were placed in hatchboxes at different locations in Myra Creek to identify any potential for toxicity. The reference site was located upstream of the discharge, and potential impact sites were located downstream of the discharge. Hatchboxes were also placed at selected locations to identify possible inputs of contaminated subsurface flows or leachates. Both lethal and sub-lethal endpoints were evaluated, including survival, hatching success, abnormalities and growth. In addition, whole body tissue concentrations of metals were evaluated.

Are the costs to meet environmental effects monitoring (EEM) benthic sample precision and accuracy criteria justified? D. Zaranko. Zaranko Environmental Assessment Services, Guelph, ON.

One of the main principles in the development of environmental effects monitoring (EEM) programs was that the monitoring technologies be cost- effective. The revised technical guidance document for EEM benthic invertebrate community surveys (Glozier et al. 2002) apparently did not use a cost-effective evaluation when recommending minimum data quality criteria. For example, the document did not correctly characterize a typical EEM benthic sample and did not account for multiple grab samples. Consequently, some of the recommended criteria cannot be realistically met in many EEM studies. Analysis of data from eight benthic projects following the EEM guidance document shows that attaining the acceptable subsampling precision and accuracy criterion of 20% is not realistic for samples with low to moderate densities of organisms. This poses a problem for clients who monitor depositional areas in streams or lake environments. If unable to attain the recommended precision and accuracy, the guidance document suggests that the entire sample be sorted. In many cases this would result in a cost of $1000 to $2000 per sample. Clearly, this is not cost effective. The question to be addressed is if a study surpasses the acceptable 20% criteria does this actually affect the conclusion as to whether or not there is a mine or pulp and paper related effect. The presentation will address this question and make recommendations for revisions to the technical guidance document.

Three cycles of environmental effects monitoring (EEM): overcoming difficulties conducting fish surveys at pulp and paper mills that discharge to marine and estuarine environments. H.M.

Dupuis[1], S.C. Courtenay[2], L.A. Rutherford[3], R. Chabot[4] and J.M. Boyd[5]. [1]Environment Canada, Environmental Protection Branch, Fredericton, NB; [2]Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB; [3]Environment Canada, Environmental Protection Branch, Dartmouth, NS; [4]Environment Canada, Environmental Protection Branch, Montreal, QC; and [5]Environment Canada, Environmental Protection Branch, Vancouver, BC.

Success of fish surveys for Canadian pulp and paper mills discharging effluent into marine and estuarine environments remains low after three cycles of Environmental Effects Monitoring (EEM). Most of these mills had difficulty completing fish surveys during the first cycle of the Pulp and Paper Effluent Regulations' EEM program. The standard fish survey did not work well due to the dynamic tidal environments and uncertainty surrounding the sustained exposure of sentinel fish to mill effluent. Success was low again during EEM Cycle 2 although better advice was provided (e.g., use of small-bodied fish as sentinels) and alternative approaches were under development for EEM (research and pilot projects). By Cycle 3, approved alternatives were available (fish mesocosms, caged bivalves) and therefore it was anticipated that more marine and estuarine mills would successfully complete fish surveys. Wild-fish surveys improved during Cycle 3, with more common use of small-bodied fish and invertebrates (e.g., gastropods, bivalves) and research to develop better endpoints for those species. Few alternatives to the standard survey were used in Cycle 3, however: only two marine mills opted to conduct caged-bivalve studies (the same number as in Cycle 2) and none conducted a mesocosm study. The reasons for the limited use of alternatives are explored, and expectations for Cycle 4 are discussed in the context of regulatory amendments in May 2004 that now require the consideration of alternative approaches to meet fish-survey requirements of the EEM program. Results of fish surveys conducted in marine and estuarine environments during Cycle 3 are also summarised.

Effect of a synthetic drilling fluid (IPAR) on blood parameters, antioxidant enzymes and peroxisome proliferation in the snow crab, *Chionoecetes opilio*. D.H. Hamoutene[1], J.F. Payne[1], C.D. Andrews[1], J.M. Guiney[1] and A. Rahimtula[2]. [1]Department of Fisheries and Oceans, Science Branch, St. John's, NL; and [2]Department of Biochemistry, Memorial University of Newfoundland, St. John's, NL.

Our study presents data on acute toxicity of IPAR; an isoalkane based synthetic fluid in use in the Newfoundland offshore. Snow crabs (*Chionoecetes opilio*) were dosed per os: 1 mL per week, 4 mL in total. Endpoints such as changes in serum composition (enzymes, proteins, and lactate), tissue levels of catalase (CAT) and glucose-6-phosphate dehydrogenase (G6PDH) as well as peroxisome proliferation were investigated. G6PDH, an enzyme involved in detoxification processes by its production of NADPH and pentose sugars did not show any changes in gills, hepatopancreas, heart or claw muscle after IPAR exposure. Similarly, CAT levels were unchanged showing no major oxidative stress. We found no significant changes in protein levels measured in supernatants prepared from hepatopancreas, heart and claw muscle. A significant protein increase in gills associated with higher blood levels of lactate was observed suggesting a potential effect on respiratory function. An increase in palmitoyl co-A oxidase was observed but was not accompanied by effects on peroxisome counts (electron microscopy). Given the high levels of synthetic fluid to which animals were exposed, results support the hypothesis that IPAR has little potential for adversely affecting the health of crabs. Considering the observed effect on gills and lactate levels, further experiments at more relevant environmental levels are necessary to assess IPAR impact on crab respiration.

Preliminary analysis of pulp and paper environmental effects monitoring (EEM) data to assess possible relationships between sublethal toxicity of effluent and effects on biota in the field. S.L. Walker[1], R.B. Lowell[2] and J.P. Sherry[3]. [1]Environment Canada, National Water Research Institute,

Gatineau, QC; [2]Environment Canada, National Water Research Institute, Saskatoon, SK; and [3]Environment Canada, National Water Research Institute, Burlington, ON.

Data from the sublethal toxicity testing of effluents may or may not be predictive of field effects. Although qualitative studies have attempted to support a predictive relationship at select sites, few quantitative studies have been undertaken to establish whether general predictive relationships exist for diverse recipient environments. Since Canada's Environmental Effects Monitoring (EEM) Program encompasses a strong field component as well as a suite of sublethal toxicity tests, the 2nd cycle data set of the pulp and paper EEM program presented an opportunity to elucidate whether relationships exist between various sublethal toxicity endpoints used in EEM and field effects that were determined in surveys of benthic invertebrate communities and fish populations. Sublethal toxicity data and key endpoints from the fish (gonad weight, liver weight, and condition) and invertebrate surveys (taxon richness and abundance) were quantitatively analyzed using simple bivariate correlation analysis. Our preliminary analysis of the data did not reveal any general relationships between the field biomonitoring and sublethal toxicity data collected under the pulp and paper EEM program.

Toxicology data supporting amendments to the Environment Canada test method using *Lemna minor*. M. Moody[1], L.N. Taylor[2] and R.P. Scoggins[2]. [1]Saskatchewan Research Council, Saskatoon, SK.; and [2]Environment Canada, Environmental Technology Centre, Ottawa, ON.

The Environment Canada *Lemna minor* growth inhibition test (EPS 1/RM/37) has primarily been in general use as a monitoring tool for assessing the sublethal toxicity of metal mining effluents, since its publication in 1999. Recent research has identified improvements in the areas of *Lemna* culture health and test procedural elements. New data supports: (i) the use of nickel sulfate rather than chromium as a reference toxicant; (ii) an improvement to the Hoagland's culture medium; and (iii) the use of black background under test containers. The inclusion of an additional *L. minor* strain (ST), and the use of Steinberg medium for the testing of chemicals are also planned. Research supporting the latter two modifications has also contributed to the development of the duckweed growth inhibition test, soon to be published by the International Standards Organization. Lastly, comparative studies of three strains of *Lemna* indicate a similar response to the reference toxicant, potassium chloride. However, *L. minor* ST was found to be four times less sensitive to the proposed reference toxicant, nickel sulphate.

Preliminary national analysis of metal mining environmental effects monitoring (EEM) sublethal toxicity data. A. Willsie, B. Ring and S.L. Walker. Environment Canada, National Water Research Institute, Gatineau, QC.

The *Metal Mining Effluent Regulations* proclaimed under the *Fisheries Act*, requires metal mines to conduct an Environmental Effects Monitoring program (EEM). To meet this objective, the EEM program requires all regulated mines to conduct biological monitoring studies which include a fish survey, a benthic invertebrate survey, as well as effluent and water quality monitoring studies. This poster focuses on the effluent sublethal toxicity testing component of EEM, which is part of the effluent and water quality monitoring studies. Toxicity tests are conducted two times a year (or once each year when regulatory conditions apply) on aliquots of samples collected for the characterization of the effluent that has potentially the most adverse impact on the environment. Tests performed on effluents discharging into the freshwater environment include larval fathead minnow *(Pimephales promelas)*growth and survival, viability of early life stages of rainbow trout (*Oncorhynchus mykiss*), *Ceriodaphnia dubia* reproduction and survival, *Lemna minor* growth inhibition and *Selenastrum capricornutum* growth inhibition. For effluents depositing into the estuarine or marine environment, tests include the larval inland silverside growth inhibition, larval topsmelt (*Atherinops affinis*) growth

and survival, echinoid fertilization and *Champia parvula* reproduction. Results presented include geometric means of inhibiting (IC25) or effective (EC25) concentrations for 25% effect as well as ranges and histograms, for most tests employed, based on data collected in 2004.

Preliminary national analysis of metal mining effluent and water quality data. B. Ring[1], A. Willsie[1], S.L. Walker[1] and M.G. Dubé[2]. [1]Environment Canada, National Water Research Institute, Gatineau, QC; and [2]Environment Canada, National Water Research Institute, Saskatoon, SK.

The *Metal Mining Effluent Regulations* (MMER), which were proclaimed in June, 2002 under the *Fisheries Act*, requires metal mines to conduct an Environmental Effects Monitoring program (EEM) to evaluate the effects of mine effluent on fish, fish habitat and the use of fisheries resources. To meet this objective, the EEM program requires all mines regulated under the MMER to conduct biological monitoring studies, which includes a fish survey and a benthic invertebrate survey, and effluent and water quality monitoring studies. This poster focuses on the effluent characterization and water quality monitoring component of EEM. Effluent characterization is conducted four times a calendar year to provide information on concentrations of compounds in effluent, which includes metals, nitrogen compounds, alkalinity and hardness. Water quality monitoring provides information on the concentration of contaminants in the exposure area as compared to the reference area and includes the same measurements as effluent characterization, as well as deleterious substances, pH, temperature and dissolved oxygen. This poster will provide an overview of the preliminary analysis of effluent and water quality data submitted by mines, which is currently being conducted by the National Environmental Effects Monitoring Office.

Development of a non-destructive technique to assess ovary size and fecundity in wild fish. B.J. Macbeth and D.M. Janz. Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, SK.

Determination of ovary size and fecundity in female fish traditionally rely on destructive (lethal) sampling, and in studies involving threatened species or requiring large sample sizes this approach is undesirable. This investigation examined the feasibility of using ultrasound images collected from live fish (prespawning female northern pike, *Esox lucius*) in the field to estimate reproductive indices. Five cross sectional ultrasound images of the left ovary were recorded at specific landmarks along the ventral surface of each fish. Assessment required five minutes per fish. Ovarian mass was determined using estimated total ovarian volume and the density of 1 mL of eggs manually expressed from each fish. After imaging, fish were euthanized and ovary mass was determined. Estimates of ovary mass, fecundity and gonadosomatic index (GSI) derived from ultrasound images were compared to those obtained from post mortem analysis. Mass and fecundity estimates were significantly correlated with post mortem measurements ($p < 0.001$, $r^2 = 0.94$ and $r^2 = 0.91$). Ovary mass and fecundity were consistently and significantly ($p < 0.001$) overestimated by mean values of 20.7% and 21.1%. GSI measurements were not significantly correlated ($p = 0.056$, $r^2 = 0.55$); GSI was overestimated by 22.6%. The accuracy of this technique may be increased by incorporating additional posterior points of imaging and a more accurate assessment of total ovarian length. In conclusion, this pilot study indicates that portable ultrasonography is a promising non-destructive technique to monitor wild fish populations in the field.

Effects of multiple mining and municipal effluents on fish from Junction Creek, Sudbury, Ontario. 1. Basic environmental effects monitoring (EEM) end-points. L.P. Weber[1], M.G. Dubé[2], C.J. Rickwood[2], K.L. Driedger[1], C. Portt[3], C. Brereton[4] and D.M. Janz[5]. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; [2]Environment Canada, National Water Research Institute,

Saskatoon, SK; [3]C. Portt and Associates, Guelph, ON; [4]INCO Ltd., Safety, Health and Environment, Copper Cliff, ON; and [5]Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, SK.

The INCO Limited mining site in Sudbury, Ontario, Canada is highly confounded with three treated metal mining-related discharges potentially affecting the Junction Creek system in a cumulative manner (Garson, Nolin Creek and Copper Cliff wastewater treatment plants (WWTP)), as well as urban run-off and municipal sewage treatment plant (STP) discharges. Adult fathead minnow (*Pimephales promelas*) and creek chub (*Semotilus atromaculatus*) were collected in May 2004 downstream of these effluent discharges and basic Environmental Effects Monitoring (EEM) end-points (metal body burdens, age, body weight, length, liver weight, gonad weight, egg size and fecundity) were measured. Metal body burdens of Cd, Cu, Rb, La, Se and Sr were elevated in one or both fish species collected downstream of WWTPs compared to reference fish, while Al, As, Co, Eu, Ga, Hg, U, V and Zn were decreased. Length-at-age was significantly lower in male and female creek chub collected downstream of Garson and Nolin Creek wastewater treatment plants (WWTPs) compared to reference sites. Liver weight was significantly increased in male fathead minnows collected downstream of Garson and Copper Cliff WWTP, while gonad weight was significantly decreased in creek chub collected downstream of Garson WWTP. Egg size was significantly decreased in both fatheads and chub downstream of all mining effluents, while fecundity was increased and decreased, respectively, in the two fish species. In conclusion, of all basic EEM end-points evaluated in this study, reproductive (egg size and fecundity) end-points were the most consistently altered in fish collected downstream of metal mining-related effluents.

Effects of multiple mining and municipal effluents on fish from Junction Creek, Sudbury, Ontario. 2. Sub-organismal responses. L.P. Weber[1], M.G. Dubé[2], C.J. Rickwood[2], K.L. Driedger[1], C. Portt[3], C.I. Brereton[4] and D.M. Janz[5]. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; [2]Environment Canada, National Water Research Institute, Saskatoon, SK; [3]C. Portt and Associates, Guelph, ON; [4]INCO Ltd., Safety, Health and Environment, Copper Cliff, ON; and [5]Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, SK.

The INCO Limited mining site in Sudbury, Ontario, Canada is highly confounded with three treated metal mining-related discharges potentially affecting the Junction Creek system in a cumulative manner (Garson, Nolin Creek and Copper Cliff wastewater treatment plants (WWTP)) as well as urban run-off and municipal sewage treatment plant (STP) discharges. Sub-organismal reproductive and bioenergetic end-points were compared in the current study to the basic Environmental Effects Monitoring (EEM) end-points (see accompanying poster) in the same adult fathead minnow (*Pimephales promelas*) and creek chub (*Semotilus atromaculatus*) collected in May 2004 from Junction Creek. This work was conducted in an effort to better understand the causal mechanisms of changes observed in the standard EEM endpoints. Higher 17β-estradiol levels were found in female fathead minnows downstream of both Copper Cliff WWTP and municipal sewage WWTP discharges (CC/STP), while male creek chub downstream of Garson and Nolin Creek WWTP discharges had lower 11-ketotestosterone levels. Histological analyses revealed that male fish collected downstream of Garson WWTP and CC/STP had immature gonads and greater testicular cell degeneration, compared to reference fish. Vitellogenin was significantly elevated in male fatheads collected downstream of CC/STP compared to reference sites. Liver triglycerides were significantly lower in fish collected downstream of CC/STP compared to reference fish, while fish liver glycogen did not differ among sites. Overall, gonadal histopathology produced the most consistent and sensitive response pattern in both fish species exposed to multiple WWTP effluents. These sub-organismal reproductive and bioenergetic end-points support causal investigation of metal mining stressors

discharged into Junction Creek.

Production water releases on the Grand Banks: potential for endocrine and pathological effects in fish. J.F. Payne[1], C.D. Andrews[1], J.M. Guiney[2] and K. Lee[3]. [1]Department of Fisheries and Oceans, Science Branch, St. John's, NL; [2]Oceans Ltd., St. John's, NL; and [3]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS.

Within the past few years, considerable interest has arisen about the potential for some chemicals to act as endocrine disruptors and possibly produce adverse effects on reproduction or other functions in fish. One of the best known examples is the "estrogenic" effect characterized by feminization of male fish with production of an intersex condition whereby male gonads are found to contain eggs as well as sperm. A prelude to the frank expression of intersex is the appearance of relatively large concentrations of yolk protein or vitellogenin in blood. A weak estrogenic potential has been linked to phenols having long alkyl chains and although the phenolic compounds common in petroleum are principally "short chain", it is not surprising that the issue of endocrine disruption would arise in connection with the discharge of production water. Norwegian researchers first put the issue in the political spotlight noting that some pure compounds possibly had the potential to produce intersex in fish. Those studies however had little ecological relevance. We have carried out a chronic toxicity study with cunners (*Tautogolabrus adspersus*) exposed to production water from the Hibernia oil field. The experiment simulated chronic conditions of exposure to which fish might be subjected within a few hundred meters of the rig site. Vitellogenin was readily induced in fish exposed to estradiol (including under winter temperatures) but no evidence was obtained for vitellogenin induction in the blood or liver tissue of cunners exposed to production water for up to 3 months. Likewise there was little evidence for histopathological abnormalities in a variety of tissues examined including liver, gill, blood and spleen. Further studies will focus on codfish (*Gadus morhua*) and plaice (*Hippoglossoides platessoides*). Such studies will provide an extra "backup" for the EEM programs on the Grand Banks where all 3 developers have included early warning indicators of effects on fish health in their monitoring programs (This study was supported in part by PERD. We also thank Rob Dunphy, Exxon-Mobil, for interest and cooperation).

Isolation and identifiction of stilbene compounds in several pulp wood species. M. Kohli, M. Comba and J. Carey. Environment Canada, National Water Research Institute, Burlington, ON.

Extracts from scotch pine (*Pinus sylvestris*) yielded three pure crystalline stilbenes. GC/MS analysis and NMR spectroscopy identified these compounds as 3,5-dimethoxypinosylvin, 3-hydroxy-5-methoxypinosylvin and 3,5-dihydroxypinosylvin. Structural confirmation was achieved by comparing these compounds with synthesized products. Nine soft and hardwood species collected from eastern and western Canada, were analyzed for the presence of these stilbene compounds. The species analyzed were scotch pine, white pine (*P. strobus*), jack pine (*P. banksiana*), balsam fir (*Abies balsamea*), black spruce (*Picea mariana*), white cedar (*Thuja occidentalis*), eastern hemlock (*Tsuga canadensis*), aspen (*Populus tremuloides*) and lodgepole pine (*P. contorta*). Only the pine species showed the presence of stilbene-like compounds. Stilbenes were not identified in balsam fir, white cedar, black spruce, hemlock and aspen. The extracts from the lodgepole pine contained the same three stilbenes as scotch pine. White pine extracts showed the presence of 3-hydroxy-5-methoxypinosylvin and 3,5-dihydrorxypinosylvin only, and at much lower concentrations than that found in scotch pine and lodgepole pine. Jack pine extracts contained similar stilbene compounds, however the substitution the of functional groups was not the same as those found for compounds in scotch pine.

Summary and trends of 2003 to 2004 metal mining environmental effects monitoring (EEM) effluent characterization and water quality monitoring data in the Prairie and Northern Region. A. Hólzapfel, M. Gray, J. Ferone and P. Siwik. Environment Canada, Environmental Protection Branch, Edmonton, AB.

Under the *Metal Mining Effluent Regulations* (MMER), metal mines discharging into the aquatic environment are required to conduct effluent characterization on each final discharge point. In addition, mines are required to monitor water quality in a reference and exposure area around each final discharge point. The first set of annual data required under the MMER was received in early 2004 for the 2003 calendar year, and 2004 annual data was received in early 2005. This poster will present a summary of the data submitted by 23 regional mines located in Manitoba, Saskatchewan, the Northwest Territories, and Nunavut. Trends and differences in effluent characterization and receiving water quality among different mining groups (i.e., Au, U and Base Metal) will be identified and discussed.

The University of Toronto culture collection of algae and cyanobacteria (UTCC): a resource centre supporting the environmental effects monitoring (EEM) program. J.C. Acreman. Department of Botany, University of Toronto, Toronto, ON.

The University of Toronto Culture Collection of Algae and Cyanobacteria (UTCC, http://www.botany.utoronto.ca/utcc) is unique in Canada. It is a service culture collection, established in 1987, focusing primarily on freshwater species, particularly those that are from areas of environmental concern. It includes species commonly used in ecotoxicity testing and those causing environmental problems in the Great Lakes and in drinking water reservoirs. UTCC serves as a resource centre for axenic cultures of algae, cyanobacteria and aquatic vascular plants used in ecotoxicity testing throughout Canada, supporting the Environmental Effects Monitoring (EEM) program. It also functions as a phycological resource centre providing workshops in methods of isolation, culture and purification, supplying sterile media, performing custom-isolation of algae and cyanobacteria and serving as a depository for research cultures and for confidential Safe-Deposit of cultures. The facility currently maintains 491 isolates, of which 357 are microalgae, 123 are cyanobacteria and 11 are aquatic vascular plants. Most of these isolates originated in central Canada; about 70% are unique to the UTCC and approximately 30% are axenic. Since inception, the collection has served close to 800 clients and annually provides about 500 research-quality cultures to users in academic, government and commercial laboratories worldwide. Approximately 57% of the requests are from academic institutions, 14% are from government labs and 29% are from commercial labs. The Natural Sciences and Engineering Research Council of Canada (NSERC) provides funding under the Major Facilities Access program for basic maintenance of the collection and user fees cover additional costs.

Linking metal bioaccumulation to effects/Faire le lin entre la bioaccumulation des métaux et leurs effets

Session co-chairs/Présidents: Warren Norwood, Richard Playle and/et Melissa Schwartz

When are metal tissue residues, or bioaccumulation data useful for effects prediction? When are they not? W.P. Norwood. Environment Canada, National Water Research Institute, Burlington, ON.

Metal bioaccumulation data can be used to predict effects. Specific examples of risk prediction

utilizing metal accumulation in test organism are presented. However, in order for metal accumulation to be truly useful the specific pattern of accumulation for that metal in a particular species must be known. As well the relationship between this pattern of accumulation and the impact on the organism must also be know such that a certain accumulation concentration is directly related to a specific amount of impact. Various patterns of accumulation are outlined and include background tissue residues, saturation kinetics, continuous accumulation and regulated tissue concentrations. Examples are provided demonstrating how some of these tissues concentration can be related to effects as well as examples in which the tissue concentration cannot be related to effects.

Are brook trout (*Salvelinus fontinalis*) stocking programs increasing the risks of mercury poisoning to wildlife in Ontario? J.W. Parks. Damsa Integrated Resources Management Inc., Thunder Bay, ON.

While threats of Hg poisoning to indigenous wildlife from aquatic organisms is receiving substantial national and international attention, there has been less focus on the role of government fish stocking programs and their potential to increase or decrease such risks. For example, the Ontario Government typically stocks more than 650 lakes annually with brook trout (*Salvelinus fontinalis*), even though results from the Ontario Sport Fish Monitoring Program indicate that mercury accumulates in many brook trout in Ontario waters to levels which exceed the Canadian Tissue Residue Guideline for the protection of mink - by up to a factor of ten. Stocked brook trout are particularly prone to predation by mink and others during spawning periods when they emigrate to small creeks to spawn - and it is these larger brook trout that contain the most Hg. Based on the precautionary principle, governments may wish to evaluate the risks of their stocking programs to wildlife, as their stocking policies may be contributing to adverse effects on wildlife. Trout stocked at larger sizes would reduce risks of Hg poisoning as concentrations in brook trout reared in aquaculture settings are substantially less than for those fish grown in the wild. Risks would also be reduced if sterile stock were utilized, as these fish are not as vulnerable to predation during spawning periods and from bioenergetic modelling considerations should accumulate less mercury than their sexually mature counterparts.

Introduction

The potential for aquatic food webs to contaminate and possibly harm fish consuming wildlife through ingestion of Hg enriched prey has and continues to receive substantial public and scientific attention. Methylmercury (MeHg) is the form of Hg that is of most concern as because it is a potent neurotoxin. It is the form most prevalent in fish and crayfish muscle. The degree of impairment to birds and wildlife from Hg exposure and the exact extent of this impairment is not easy to ascertain. There are difficulties in measuring and ascertaining wild population effects to Hg poisoning per se when mortality may well be the result of multiple stressors, of which Hg is only one.

Environment Canada (2003) has noted that Hg exists in Canada at levels that are causing deleterious impacts on wildlife. Further, Hg levels in fish are high enough to put wildlife such as loons, kingfishers, herons, osprey and mink at risk of adverse health effects. Some of these risks were identified for Ontario (Kent et al. 1998). Reproduction of loons in Ontario appears at risk. Concentrations in prey in a significant number of Ontario lakes likely have mercury concentrations above the threshold for reproductive impairment (Scheuhammer and Blancher 1994).

Otters may be at risk to reduced survivorship in Ontario. Mierle et al. (2000) observed that otter survivorship appeared to decrease as Hg levels in their hair, which reflect dietary exposure, increased.

The mean age of otters in low Hg townships was nearly twice that observed for otters in high Hg townships. Later work by Klenavic (2004) also shows that there are no long lived otters and mink with high Hg levels in brains. This work also suggests possible adverse population impacts of these animals to exposure to MeHg.

Assessing risk of Hg poisoning to wildlife

The risk of Hg poisoning to birds and wildlife from consuming aquatic organisms with elevated levels of MeHg was assessed by Environment Canada (2002) in their document "Canadian Tissue Residue Guidelines (CTRG) for the Protection of Consumers of Aquatic Life: Methylmercury". This report establishes species specific upper limits for Hg levels in the diet of various consumers of aquatic organisms based upon the ecotoxicology of MeHg to the animal or bird in question. The goal of the CTRG values is to determine a concentration of MeHg when consumed by wildlife that will not result in adverse effects.

The report recommends that to protect Canadian wildlife that consume fish or shellfish from any toxicological effects of MeHg, aquatic biota should contain no more than 33 $\mu g \cdot kg^{-1}$ MeHg on a wet weight basis. This guideline of 33 $\mu g \cdot kg^{-1}$ is designed to protect the most sensitive species in Canada, the Wilson's storm petrel (*Oceanites oceanicus*). As this bird does not commonly inhabit Ontario, guidelines for more appropriate species should be viewed and diet guidelines for these species are slightly less restrictive.

For birds and animals common to Ontario, such as the common tern (*Sterna hirundo*), concentrations in aquatic prey organisms should not exceed 0.051 $\mu g \cdot kg^{-1}$, for the belted kingfisher (*Megaceryle alcyon*), 0.062 $\mu g \cdot kg^{-1}$; for the female wood duck (*Aix sponsa*), 0.089 $\mu g \cdot kg^{-1}$; for the female herring gull (*Larus argentatus*), 0.107 $\mu g \cdot kg^{-1}$; for the female common merganser (*Mergus merganser*), 0.115 $\mu g \cdot kg^{-1}$ for the common loon (*Gavia immer*), 0.172 $\mu g \cdot kg^{-1}$; for the bald eagle (*Haliaeetus leucocephalus*), 0.282 $\mu g \cdot kg^{-1}$; for the Osprey (*Pandoin haliaetus*), 0.155 $\mu g \cdot kg^{-1}$; for the female great blue heron (*Ardea herodias*), 0.141 $\mu g \cdot kg^{-1}$; for female mink (*Mustela vison*) it is 0.092 $\mu g \cdot kg^{-1}$; and for the river otter (*Lutra canadensis*), 0.220 $\mu g \cdot kg^{-1}$. The criterion is different for each species owing to differences in metabolism, diet, and vulnerably to Hg poisoning.

The vast majority of waters in Ontario have not been monitored for Hg concentrations in prey. There are many places, however where Hg concentrations in prey fish such as perch or other aquatic food items such as crayfish exceed the CTRG for sensitive wildlife by up to ten times or more (Allard and Stokes 1989, Suns and Hitchin 1990, Parks et al. 1991, Swanson et al. 2003). Brook trout can be a prey item for many wildlife including kingfishers, mergansers, loons, herons, mink and otters. Insights into the potential for brook trout in Ontario waters to pose risks of mercury poisoning to these fish consuming wildlife can be gained by comparing concentrations of Hg in brook trout in Ontario with Environment Canada (2002).

Hg concentrations in brook trout were obtained for over 70 sites (Fig. 1) from the Ontario Sport Fish monitoring program conducted by the Ontario Ministries of the Environment, and Natural Resources (2005). It contains over 150,000 records from over 1300 water bodies, and has had rigorous QA/QC protocols for Hg measurements from its inception. Hg concentrations for 1281 brook trout ranged from 0.01 to 1.2 $\mu g \cdot kg^{-1}$ with higher concentrations in larger fish (Fig. 2). While there is relatively little data for the smaller size trout which would make up most of the prey items for most wildlife (< 15 to 20 cm), the available data do indicate that mercury levels in trout in Ontario can exceed CTRG and thus pose risks to wildlife (Figs. 3 and 4). Levels of Hg in bigger trout may have relevance to otters and mink, which are fully capable of killing and consuming 30 to 40 cm trout or larger if they are vulnerable to capture. Concentrations of Hg in these larger brook trout can exceed 1 $\mu g \cdot kg^{-1}$, a level shown to be lethal in toxicological studies with mink (Wren et al. 1987).

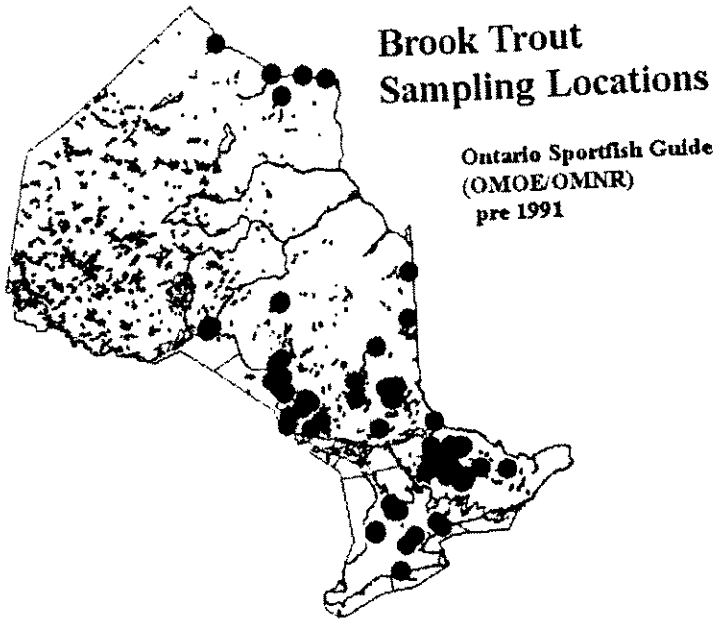

Figure 1. Brook trout sampling locations for the Ontario Sport Fish monitoring program.

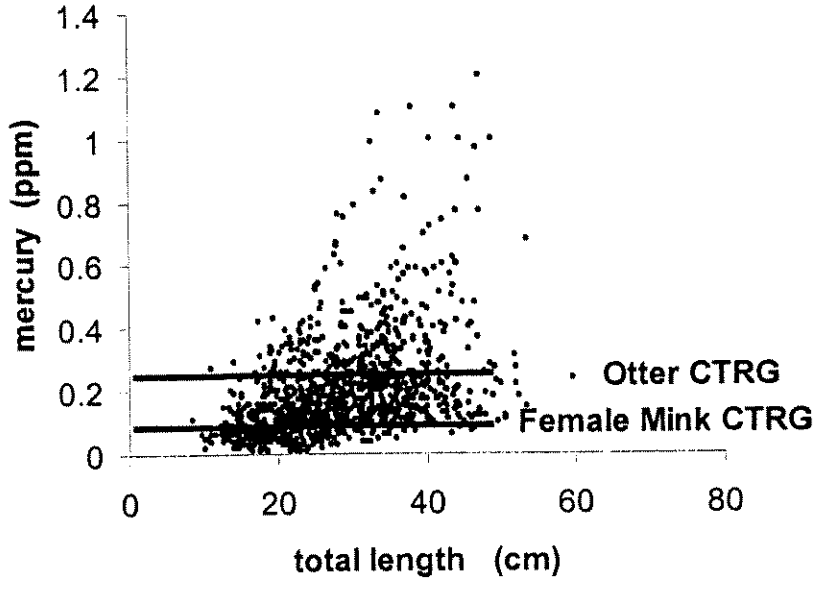

Figure 2. Mercury concentrations in brook trout (n=1281) in Ontario waters, with Canadian Tissue Residue Guidelines (CTRG) for otter and female mink.

The results for brook trout (Figs. 2 to 4) may contain data for stocked lakes, but no attempt has been made here to identify results for stocked versus indigenous fish, merely to show that Hg levels can accumulate to levels in trout that pose a risk to fish consuming wildlife. It would not be surprising if these results did include some stocked fish as the Ministry of Natural Resources stock over 650 lakes a year with brook trout (S.J. Kerr, Ontario Ministry of Natural Resources, pers. comm.) These stocked trout range in size from fry to adult brood stock that is no longer needed by the Ministry of Natural Resources.

Why stocking programs may contribute to risks of mercury poisoning to wildlife

When stocked, brook trout likely cause little risk to wildlife consumers, independent of the size at which they are stocked because Hg concentrations in trout in hatchery or aquaculture operations typically have low concentrations of Hg - even in larger fish. Damsa grew brook trout in a hatchery in excess of 50 cm that had Hg concentrations less than 0.033 $\mu g \cdot kg^{-1}$, the lowest criterion established by Environment Canada to protect all wildlife consumers. These results are consistent with those released by the Ontario Ministry of Agriculture and Food for a study on farm raised fish in Ontario. For 59 samples of rainbow trout, Hg concentrations were typically less than the 0.01 $\mu g \cdot kg^{-1}$ with a range of < 0.01 to 0.07 $\mu g \cdot kg^{-1}$ (Cassidy et al 2003). However the longer the trout survive in stocked waters, the more their body burdens will reflect exposure to MeHg in the ambient environment. Fry will respond most quickly; when they grow to the size of prey for most predators, they will reflect over 90% of the concentrations of indigenous trout. Large brood stock, on the other hand, will respond the slowest and may not attain substantial increases in Hg concentrations before they are angled or die from natural factors. Other sizes stocked will fall somewhere in between.

Although Hg levels in fish in Ontario are amongst some of the highest in the country (Environment Canada 2002), the elevated tissue levels are not necessarily associated with point source or historical loadings of mercury into the aquatic environment. Many of these fisheries are associated with biogeochemical and/or hydrological conditions that enhance Hg bioaccumulation in fish independent of Hg loadings. Lakes with the potential for biota to accumulate high Hg concentrations have certain characteristics including low pH, high dissolved organic carbon concentrations and low productivity (Evers 2005).

Brook trout are particularly adaptable to these same waters as they have a high tolerance to acid conditions. They also prefer cooler waters, which are enhanced by elevated dissolved organic carbon concentrations. Dissolved organic carbon effectively limits the depths to which light penetrates and warms, thereby increasing the depth of the hypolimnion and the quantity of cooler water available for trout habitat during summer (Schindler and Gunn 2004). Brook trout also prefer low productivity systems, as it is productivity that frequently limit over winter survival by lowering the dissolved oxygen levels to lethal conditions. For these reasons it would not be surprising to find brook trout stocked in some of these ecosystems that have a known propensity to produce biota with high mercury concentrations.

Some stocked brook trout will become vulnerable to predation at much larger sizes than normally considered for prey. They are particularly vulnerable at larger sizes during spawning periods. Brook trout stocked in Ontario typically do not successfully reproduce, as there are poor or no suitable spawning locations in majority of the lakes in which they are stocked. During spawning periods in these types of lakes, stocked fish may emigrate to spawn in lake outflows (Josephson and Youngs 1996, Josephson et al. 2001).

Outflows in Ontario brook trout lakes are usually quite small, reflective of small headwater systems that provide much if not most brook trout habitat in lakes in Ontario and most often lake levels are beaver dam controlled. Trout emigrating to spawn in such small creeks are particularly vulnerable to

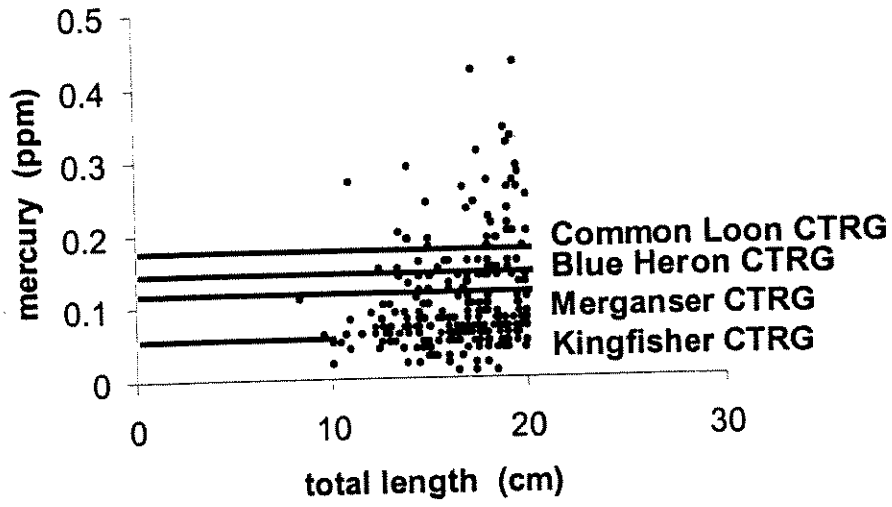

Figure 3 Mercury concentrations in brook trout (< 20 cm) in Ontario waters with Canadian Tissue Residue Guidelines (CTRG) for selected birds.

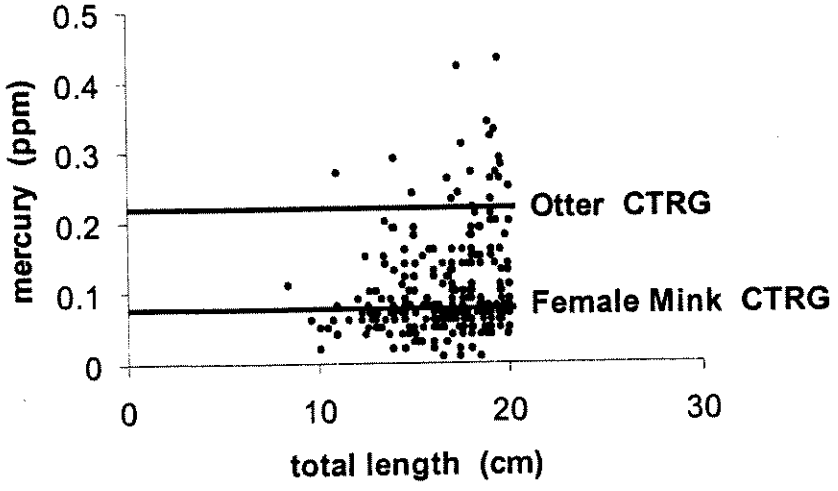

Figure 4 Mercury concentrations in brook trout (< 20 cm) in Ontario waters with Canadian Tissue Residue Guidelines (CTRG) for selected mammals.

predation; even more so when they are unable to return to the lake after spawning due to obstruction by the beaver dam. These larger sexually mature brook trout are usually predated within two to three weeks (D.C. Josephson pers. comm.). Both otter and mink are capable of capturing and killing sexually mature trout in the 30 to 40 cm size range or larger as many fish farmers will attest, provided that the predators can access the trout. Thus in vulnerable spawning conditions these larger trout with the highest Hg concentrations can also be prey to otters and mink.

Future considerations

The available evidence suggests that brook trout in Ontario waters have Hg concentrations that can be of concern to wildlife consumers and further that stocked fish may be contributing to these concerns. As the province ascribes to the precautionary principle, the regulatory agencies may wish to further assess the risk of mercury poisoning to wildlife, as they may, at present, be deliberately, though inadvertently, creating additional risks through their stocking programs.

Such assessments could include the collection of additional data on Hg levels in stocked brook trout lakes, particularly those lakes with biogeochemical characteristics that have a known potential to create high Hg fisheries. Should high risks be identified, steps could be undertaken to reduce such risks through modifications to size of fish at stocking, stocking rates, and the possible inclusion of sterile fish. According to bioenergetic modelling considerations, sterile trout would accumulate lower levels of Hg than their sexually mature counterparts (Rodgers 1994). Additionally such fish would be less vulnerable to predation during spawning periods as they do not emigrate to spawn (Warrilow et al. 1997). Other adjustments to the ecosystem to reduce Hg bioaccumulation could include increasing productivity, or other biogeochemical alterations that reduce levels of methylmercury to which fish, aquatic organisms, and ultimately wildlife, are exposed.

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Tissue metal and metallothionein concentrations as biomarkers of cadmium exposure and predictors of toxic effects in benthic invertebrates. P.L. Gillis[1], T.B. Reynoldson[2] and D.G. Dixon[3]. [1]Department of Biology, McMaster University, Hamilton, ON; [2]Environment Canada, National Water Research Institute, Wolfville, NS; and [3]Department of Biology, University of Waterloo, Waterloo, ON.

The relationships between Cd accumulation, induction of a metallothionein-like protein (MTLP) and the effects of Cd exposure were investigated using the oligochaete, *Tubifex tubifex* and the midge, *Chironomus riparius*. In laboratory exposures with Cd-enriched, field-collected sediment, the sub-cellular endpoints of tissue Cd and MTLP concentration both exhibited a concentration-response relationship with metal exposure. After reaching a threshold of Cd exposure, the whole-body endpoints of reproductive output in *T. tubifex* and growth in *C. riparius* declined significantly. Significant changes in the sub-cellular endpoints were observed at Cd concentrations much lower than was required for significant changes in the whole-body endpoints, for example in *T. tubifex* the MTLP concentration was significantly higher than the control level after exposure to

the low end of the exposure range whereas a significant decline in the reproduction was not detected until the Cd exposure had increased by 4 fold. Significant increases in tissue Cd and MTLP could be measured within 8 h of exposure, whereas changes in reproduction and growth could not be determined until completion of longer term exposures (*T. tubifex* 28 d, *C. riparius* 10 d). Further investigation into the natural variation of MTLP concentrations in the absence of metal exposure found it to be a robust biomarker which was not influenced by life stage of the animal or induced by environmental stressors such as food limitation and excess handling. Once the relationships between tissue concentrations of metals and/or other toxicant-specific inducible biomarkers such as MTLP and the measurable toxic effects have been established, then these sub-cellular endpoints can be used to predict if ecologically relevant effects would be expected to occur at a given tissue concentration. Correlating sensitive endpoints such as these to effects at the individual or population level is a simple yet powerful tool for assessing the risk associated with metal exposure.

Predicting effects from the bioaccumulation of 10 metals with a Metal Effects Addition Model (MEAM). W.P. Norwood[1], D.G. Dixon[1] and U. Borgmann[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Environment Canada, National Water Research Institute, Burlington, ON.

Multiple metals are often elevated simultaneously in the sediments and water at sites of contamination. A "Metal Effects Addition Model" (MEAM) is outlined and used to evaluate the impact of 9 metals (Cd, Co, Cr, Cu, Mn, Ni, Pb, Tl and Zn) and the semi-metal (As) at a number of contaminated sites. One week bioaccumulation/bioavailability tests with *Hyalella azteca* are used to predict chronic survival and are compared to chronic toxicity tests performed on these sites. As well, the bioaccumulation and bioavailability data can be used for Toxic Identification and Evaluation of the sites.

Pathways of metal uptake and toxicity in fish: putting physiology into environmental regulations. C.M. Wood. Department of Biology, McMaster University, Hamilton, ON.

Historically, ambient water quality criteria for metals have been based simply on total or dissolved metal concentrations. However, we now know that bioavailability and toxicity of waterborne metals vary greatly with water chemistry characteristics that are site-specific - such as hardness, specific ion levels, pH, alkalinity, and dissolved organic carbon. An understanding of both aquatic geochemistry and the physiology of the gills is critical to understanding the influence of these modifying factors. For example, Ag and Cu target branchial Na^+ transport mechanisms, while Zn, Ca, Co, and Pb target branchial $Ca2^+$ transport mechanisms. The routes of normal metal uptake and bioaccumulation, at least in part, appear to be the same as the pathways of toxicity, and we now understand much about the particular cells and processes involved. Based on this knowledge, we have contributed to the development of the Biotic Ligand Model (BLM), a computational framework for the prediction of acute metal toxicity in any water quality. The BLM puts this physiological knowledge into environmental regulations which are cognizant of receiving water chemistry, and thereby both protective and socio-economically realistic. The BLM has been accepted as a regulatory procedure by the U.S. EPA, and is in various stages of study or approval by other agencies around the world. Our recent research has focused on extending the BLM approach to more sensitive sentinel organisms, to the prediction of chronic endpoints, and to the prediction of dietary metal toxicity. Already it is clear that an understanding of gastro-intestinal physiology, and its interaction with gill physiology, will be critical for both of the latter goals. (Supported by NSERC CRD, ICA, CDA, NiPERA, ILZRO, Teck Cominco, Noranda- Falconbridge, and INCO).

A biotic ligand model approach to copper toxicity in tropical freshwater zebrafish (*Danio rerio*). N. Franklin, G. McClelland and C.M. Wood. Department of Biology, McMaster University, Hamilton, ON.

The Biotic Ligand Model (BLM) is a promising new approach for deriving site-specific water quality criteria, based on the metal concentration and on the degrees of complexation and competition with environmental factors. Currently however, there are a limited number of organisms for which acute BLM's exist. In the present study, the effects of a wide range of key water quality parameters (Na^+, Ca^{2+}, Mg^{2+}, K^+, pH, DOC) on gill Cu binding and acute toxicity are being studied using the freshwater teleost fish, zebrafish (*Danio rerio*). The zebrafish answers the need for a tropical species, is softwater tolerant, and offers the additional advantage of a well-characterized developmental biology and publicly available genomic information. Cu accumulation on the gill at 25 $\mu g \cdot L^{-1}$ dissolved Cu (in softwater) decreased with increasing water Na^+ and Ca^{2+} levels. Gill Cu was approximately 70% lower at 10 mM Na^+ (4.4 $nmol \cdot g^{-1}$), and 80% lower at 3.3 mM Ca^{2+} (2.9 $nmol \cdot g^{-1}$ after 3 h exposure (using ^{64}Cu) compared to the softwater control (15.6 $nmol \cdot g^{-1}$). Observed changes in the binding profile correlated with changes in acute Cu toxicity (96 h LC50), with increased concentrations of Na^+ (10 mM) and Ca^{2+} (3.3 mM) resulting in a reduction of acute toxicity by a factor of 30 and > 15, respectively. The characterization of the effects of water chemistry on Cu gill-binding properties in the zebrafish is an important step towards extending the acute Cu-BLM for fish. (Funded by ILZRO, ICA, CDA, NiPERA, Noranda-Falconbridge, Teck Cominco, INCO, and an NSERC CRD grant).

Implications of sublethal copper exposure on the ammonia tolerance of the goldfish (*Carassius auratus*). W. Etches, J.S. Klinck, R.C. Playle and M.P. Wilkie. Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Ammonia may be encountered by fishes in the wild due to run-off from agricultural operations, discharge of sewage effluents, or leachate from landfills. In aquaculture operations or aquaria, ammonia may arise from nitrogenous waste excretion by resident fishes or the degradation of uneaten food. Cu may originate from industrial, mining and municipal effluents, but it is also found in many algicides, fungicides and anti-fouling agents used in aquaculture operations. The possibility therefore exists that fishes could be simultaneously exposed to Cu and ammonia in natural or aquacultural settings. In the present study, the acute toxicity of ammonia to goldfish (*Carassius auratus*) was determined at total ammonia concentrations ($T_{Amm} = [NH_4^+] + [NH_3]$) of 0, 0.375, 0.750, 1.500, 3.125, 6.250, 12.500, and 25.000 $mmol \cdot L^{-1}$ in Cu-free or Cu-contaminated ($[Cu^{2+}] = 0.74 \pm 0.04$ $\mu mol \cdot L^{-1}$) hard water ($[Ca^{2+}] = 1.0$ - 1.5 $mmol \cdot L^{-1}$; pH 7.8). In freshwater fishes, ammonia toxicity is mainly related to the environmental NH_3 concentration, rather than NH_{4+}, because ammonia mainly permeates the fish gill as un-ionized NH_3. Goldfish held in Cu-contaminated water had a T_{Amm} 96 h LC50 of 2.69 $mmol \cdot L^{-1}$, which equates to an NH_3 96 h LC50 of 100 $\mu mol \cdot L^{-1}$. In Cu-free water, however, the 96 h LC50 for T_{Amm} was 8.44 $mmol \cdot L^{-1}$, with a corresponding NH_3 96 h LC50 of 292 $\mu mol \cdot L^{-1}$. We conclude that sub-lethal concentrations of Cu increase the susceptibility of goldfish to ammonia toxicity. In addition, the acute NH_3 toxicity data in Cu-free water reveals goldfish as one of the most ammonia tolerant freshwater fishes known.

Development of a field-based kinetic model for cadmium bioaccumulation in indigenous yellow perch (*Perca flavescens*). L.D. Kraemer, P.G.C. Campbell and L. Hare. Institut National de la Recherche Scientifique – Eau, Terre et Environnement, Université du Québec, Sainte-Foy, Qc.

A key knowledge gap in environmental risk assessment of metals is the relative importance of dietary and aqueous metals as vectors for metal accumulation in fish. To address this knowledge gap,

we used a series of field transplantation and caging studies to develop a Cd bioaccumulation model for indigenous populations of yellow perch (*Perca flavescens*). The bioaccumulation model was designed to examine parameters important for dietary uptake (e.g., ingestion rate, assimilation efficiency) separately from parameters important for aqueous uptake (e.g., aqueous metal concentrations). The model was then used to predict the relative importance of dietary and aqueous Cd as routes of exposure for populations of yellow perch living in lakes with different degrees of Cd contamination. By testing this model in yellow perch from various environments, we demonstrated that by just taking aqueous Cd^{2+} concentrations into account, the model accurately predicted hepatic Cd concentrations in fish from lakes with elevated aqueous Cd^{2+} concentrations. The contribution of dietary Cd was more important in fish from reference and moderately contaminated lakes, and this importance decreased with increasing aqueous Cd^{2+} concentrations. From the perspective of risk assessment, these results imply that aqueous Cd^{2+} concentrations are the major factor influencing accumulation in perch from Cd-contaminated lakes. Predicting Cd concentrations in fish from reference and intermediate lakes is more difficult as the diet plays a larger role.

Influence of acclimation to copper or cadmium on the bioaccumulation of copper and cadmium. J.C. McGeer[1], S. Nadella[2] and C.M. Wood[2]. [1]Natural Resources Canada, CANMET, Ottawa, ON; and [2]Department of Biology, McMaster University, Hamilton, ON.

The understanding of the potential impacts of chronic metal exposure is complicated by the fact that responses of fish to long term metal exposure can be variable, for example, gill metal burden can change considerably, and as well, many metals induce acclimation. This study examines the influences of pre-exposure to chronic sublethal waterborne Cu or Cd on the subsequent uptake and distribution of Cu and Cd in rainbow trout (*Oncorhynchus mykiss*). Fish were exposed to either 3 $\mu g \cdot L^{-1}$ Cd or 75 $\mu g \cdot L^{-1}$ Cu for one month with minimal mortality and no effect on growth. Chronic sublethal exposure to metal resulted in acclimation (resistance to acute challenge) to the metal of exposure as well as cross acclimation to the other metal. Exposure also resulted in significant accumulations of metal (either Cd or Cu) in the gills, kidney, liver. Accumulations of Cd were greatest gill while those for Cu were highest in the liver. Following a month of exposure metal uptake into the gills, blood, liver, kidney, gastrointestinal tract and whole body was studied over time using radioisotopes (^{109}Cd or ^{64}Cu). Acclimation to Cd resulted in a reduction in the rate of Cd uptake into the gills and whole body compared to uptake rates of naive fish exposed to 3 $\mu g \cdot L^{-1}$ Cd. When challenged at the higher level of 10 $\mu g \cdot L^{-1}$ Cd, acclimated fish had significantly higher rates of gill Cd uptake and fish previous exposure to Cu had similarly enhanced Cd uptake. In addition, chronic metal exposure resulted in altered routing of new metal through different body compartments. The results illustrate the changes in metal uptake and internal distribution that occur during chronic exposure and help to build an understanding of the physiology of acclimation responses.

Total mercury of amphipoda taken from stomach contents of yellow perch (*Perca flavescens*) at Cornwall, ON – Area of Concern. L.E. Yanch, P.V. Hodson and L.M. Campbell. Department of Biology, Queen's University, Kingston, ON.

After decades of exposure to industrial pollutants, sediments along depositional zones at the St. Lawrence River waterfront at Cornwall, Ontario are highly contaminated with Hg. The popular sport-fish species, walleye (*Sander vitreus*) and yellow perch (*Perca flavescens*), are significantly more contaminated relative to other top consumer fish from other sites. Hg concentrations in walleye, standardized to 45 cm, in Lake St. Francis (downstream from Cornwall) between 1998 and 2000 were 0.59 $mg \cdot kg^{-1}$ wet wt; exceeding the Ministry of Environment guideline of 0.45 $mg \cdot kg^{-1}$ wet wt. Preliminary evidence indicates that differences in trophic chain length leading to yellow perch, among

zones do not exist, so food chain length variation cannot explain the differential in Hg concentrations in yellow perch from different zones within the St. Lawrence River. Stomach content analyses indicate yellow perch are highly selective and proportions of dietary items found in stomach samples do not reflect actual proportions of the same items in the field. Hence, prey selection, but not food chain length, may be an important factor in explaining variation in Hg burdens in yellow perch. Since walleye have been found to be more contaminated than yellow perch, it is important to determine the influence of trophic food chain length and dietary selection on Hg bioaccumulation. Presently, we are investigating the bioaccumulation of methylmecury transferred through the aquatic food chain to walleye. Fish stomach content identification and analysis, as well as trophic level assignment through the use of stable nitrogen and carbon isotope analysis will be used.

Olfactory sensitivity of wild yellow perch (*Perca flavescens*) from along a metal contamination gradient. W.W. Green[1], R.S. Mirza[2], C.M. Wood[1] and G.G. Pyle[2]. [1]Department of Biology, McMaster University, Hamilton, ON: and [2]Department of Biology, Nipissing University, North Bay, ON.

In the predominately industrial mining areas of northern Ontario, common constituents of industrial pollution include a variety of metals that have been shown to negatively impact the olfactory abilities of fishes. Fishes use olfaction to detect chemical cues that influence a variety of behaviours including foraging, reproduction, and assessment of predation risk. Predator avoidance is mediated by the detection of chemical alarm cues by conspecifics and results in the occurrence of anti-predator behaviours such as area avoidance, shelter seeking, increased shoal cohesion, decreased activity, and decreased foraging. In percids, specifically yellow perch (*Perca flavescens*), chemical alarm cues are released when damage to the skin occurs such as during a predation event. Previous field studies in the North Bay - Sudbury area involving another percid, the Iowa darter (*Etheostoma exile*), have shown that metals can impair a fish's ability to detect chemical alarm cues. In this study, yellow perch were collected from five lakes along a metal contamination gradient in the North Bay – Sudbury region and their olfactory sensitivity to conspecific alarm cue was tested using an electro-olfactogram apparatus. If yellow perch are unable to detect chemical alarm cues they are at greater risk of being preyed upon. Furthermore, it is possible that if yellow perch cannot detect alarm cues using olfaction then they may also be unable to detect food or identify appropriate mates. This impairment of chemosensation may cause large-scale ecological perturbations such as population declines in yellow perch and an overall decrease in the aquatic ecosystems health. (Funded by MITHE-RN and NSERC).

Protective effects of tea against metals binding to gills of rainbow trout (*Oncorhynchus mykiss*). J.S. Klinck, G. Ivanis, A.R. Winter and R.C. Playle. Department of Biology, Wilfrid Laurier University, Waterloo, ON.

In some northern communities, individuals exposed to high amounts of mercury from contaminated fish, exhibit low levels of mercury in blood and hair samples. This discrepancy could be the result of people drinking tea with their fish meals. It has been hypothesized that flavonoids in tea act as strong metal-chelating agents, thus making them less available to be absorbed by the body. To investigate the protective effects of tea, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to four different heavy metals in the presence of Orange Pekoe tea and natural organic matter (NOM) collected from Luther Marsh. Previous experiments showed that NOM from Luther Marsh greatly reduced metal accumulation by trout gills, thus was used as a comparison. The quality of NOM appears to affect its binding strength for some metals, but not for others. Luther Marsh (LM) is optically dark (SAC_{340}= 32.2) compared to the tea (SAC_{340}= 16.8) and their excitation-emission matrices are quite different. Tea binds Cu and Hg as well as Luther Marsh NOM, but less well for

Pb and Cd, as judged by metal binding to trout gills. In all cases tea and NOM reduced the accumulation of metals at pH 7. Further experiments are being performed with juvenile trout to evaluate the chelating activity of tea.

Arsenic concentration and speciation in fish from Back Bay near Yellowknife, NT. S. De Rosemond[1], K. Liber[1], Q. Xie[2] and M. Liskowich[3]. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; [2]Water Quality Centre, Trent University, Peterborough, ON; and [3]Indian and Northern Affairs Canada, Northwest Territories Region, Yellowknife, NT.

The objective of this study was to determine the concentrations of total As and different As species in fish tissues so that a more complete risk assessment of human exposure to arsenic through consumption of fish from Back Bay in Great Slave Lake, NT, could be performed. To this end, the concentrations of total As and five As species [As(III), As(V), monomethylarsonic acid (MMA), dimethylarsenic acid (DMA), and arsenobetaine (AsB)], were measured in muscle (fillet), liver and the gastrointestinal tract (GIT) of lake whitefish (*Coregonus clupeaformis*), walleye (*Sander vitreus*), northern pike (*Esox lucius*), white sucker (*Catostomus commersoni*) and longnose sucker (*Catostomus catostomus*). Across fish species, the mean (± SD) concentration of total As in the muscle, liver and GIT were 0.84 ± 0.43, 1.24 ± 1.15, and 3.28 ± 4.24 $mg \cdot kg^{-1}$ dry wt, respectively. Among fish species, white sucker had significantly higher total As concentrations in the GIT than walleye northern pike and lake whitefish, and higher total As concentrations in the liver than lake whitefish. The concentration of total As in muscle among fish species was not significantly different. In all fish species, only a very small portion (< 4%) of the total As identified in the muscle was inorganic As [As(III) and As(V)]; inorganic species were considered the most toxic forms of As and are of toxicological concern when conducting a human health risk assessment. The majority (> 50 %) of organic As in most of the tissues from fish caught in Back Bay was not analytically identified. Evidence from the literature suggests that most of these other organic As species could have been trimethylated As compounds; however, further analytical work would need to be performed to verify this hypothesis.

Impact of organic matter source on metal binding and toxicity: fluorescence spectroscopy and mixture resolution. S. Smith[1] and R.C. Playle[2]. [1]Department of Chemistry, Wilfrid Laurier University, Waterloo, ON; and [2]Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Metal binding to natural organic matter (NOM) can modify the toxicity of metals in aqueous systems. The source of organic matter influences the form of metals in solution and thus the potential toxic effects. Fluorescence spectroscopy is a powerful technique to characterize NOM from different source and this characterization can potentially be utilized to predict metal binding and metal toxicity. In terms of a Biotic Ligand Model framework (BLM) this fluorescence characterization method can be viewed as a quality factor rather than a quantity factor. Fluorescence excitation versus emission scans were obtained for six organic matter samples obtained from a variety of sources, including marshes, algae-rich ponds, Lake Ontario, and sewage effluent. The fluorescence scans were then resolved into components using both SIMPLISMA and PARAFAC analysis techniques. Fluorescence spectroscopy measures both light scattering and fluorescence. A two dimensional interpolation to replace non-fluorescent data is performed prior to SIMPLISMA analysis. An advantage of PARAFAC analysis is that interpolation is not necessary. Both spectral resolution techniques gave a similar set of four components that describe the complete dataset. These include two amino-acid like fluorescent components, corresponding to tyrosine and tryptophane-like fluorescence, and, two longer wavelength components corresponding to humic and fulvic acid species. The integrated volume corresponding to each component is proportional to its concentration. These component profiles can then be used

as fingerprints for organic matter characterization. These organic matter source-dependent fingerprints are compared to existing metal toxicity data for these same samples.

Multiple metals binding to gills of rainbow trout (*Oncorhynchus mykiss*). A.R. Winter[1], J.S. Klinck[1] and R.C. Playle[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Department of Biology, Wilfrid Laurier University, Waterloo, ON.

The effects of metal binding to the gills of fish are often investigated with solutions of individual metals, but metal mixtures are common. It is important therefore to understand how metals in mixtures interact with each other and with aquatic organisms like fish. Multiple metal-gill modeling using the toxic unit concept indicates that metals with the same toxic actions (e.g., Pb and Cd acting at Ca uptake sites) will exert toxicity in a strictly additive fashion if the concentrations of the two metals sum to one toxic unit. Below one toxic unit the metals will be more than strictly additive, due to the non-linear nature of the models. Above one toxic unit the metals will be less than strictly additive, due to competition between the metals. Our research tests the model against reality by exposing about 5 g juvenile rainbow trout (*Oncorhynchus mykiss*) to mixtures of Pb and Cd (at 0.8, 1.5, 2.2, and 3.0 μM each). Results to date show the non-linear nature of trout gills accumulating metals, and approximate additivity at lower metal concentrations (1.5 μM Pb + 1.5 μM Cd). There is some evidence of competitive effects at the highest concentrations, because gill Cd for trout exposed to mixture treatments are lower than that for trout exposed to Cd alone, while gill Pb remains high. Continuing work is investigating the bioaccumulation and partitioning of Cd and Pb within the body of about 50 g rainbow trout when exposed to mixtures of these two metals.

Nearshore contaminated sediment investigations at Britannia Beach, British Columbia. M.E. Hagen[1], A. Yao[2], R. McCandless[2], R. More[2] and A.G. Colodey[2]. [1]Environment Canada, Environmental Protection Branch, Vancouver, BC; and [2]Environment Canada, Environmental Conservation Branch, Vancouver, BC.

The Britannia copper mine, 45 km north of Vancouver, BC, discharged about 40 million tonnes of mine tailings into Howe Sound between 1905 and 1974. The mine will continue to discharge metals-contaminated drainage until a wastewater treatment plant is completed in 2006. Environment Canada (EC) investigated the shallow, nearshore marine environment at Britannia Beach during 2001 and 2002. Bottom habitat at depths of less than about twenty meters is the most biologically productive zone and also the maximum practicable depth for any cost-effective sediment remediation, should any be necessary. Multiple lines of evidence were used to obtain a more complete understanding of the nearshore benthic environment than provided through traditional sediment sampling methodologies. EC conducted or contracted six surveys between April 2001 and March 2002. Video was used to map seabed substrate type, morphology, and the types and distribution of organisms. More traditional studies included analyses for sediment particle size and sediment chemistry (including measures of bioavailability), water and sediment bioassays, bioaccumulation (both *in situ* and in amphipods exposed in the lab), and benthic invertebrate community analysis. Integrating the lines of evidence suggested that the Britannia Beach study area can be divided into four sub-areas which range from background conditions to areas of severe impact. The priority for management action is an area north of Britannia Creek and the deep outfall stretching about 1100 m alongshore where conclusive sediment-related effects were demonstrated. Sediment metals were high, bioavailable, induced toxicity in bioassays, and the abundance and diversity of benthic invertebrate communities was reduced. We recommend re-assessment of the benthic community and the impact of sediment contamination after the proposed mine water treatment plant has operated for at least three years because mine water deposits may be a major contributor to the impacts found.

Oil sands assessment/Évaluation des sables bitumineux

Session co-chairs/Présidents: George Dixon and/et Michael van der Heuvel

Using stable isotopes to trace the bacterial degradation of naphthenic acids. P.P. Videla, A.J. Farwell, B.J. Butler and D.G. Dixon. Department of Biology, University of Waterloo, Waterloo, ON.

Large amounts of process-affected water (tailings) are generated from the extraction of bitumen from the oil sands in Alberta. Prior to reclamation, the tailings are placed into settling basins, and consist of sand, clay, unrecovered bitumen as well as large quantities of naphthenic acids (NAs) and polycyclic aromatic hydrocarbons (PAHs). NAs are complex mixtures of carboxylic acids that have been analytically characterized by C numbers (C5 to C33) and Z families (Z=0, linear; Z=-12, 6 rings). Acute toxicity to aquatic organisms has been traced to the NAs, which to some extent are biodegradable thereby potentially reducing their toxicity. Biodegradation of NAs in reclaimed aquatic environments is important in determining the structure and function of the mature aquatic ecosystems. To improve our understanding of energy sources and trophic level interactions, stable carbon and nitrogen isotopes have been analyzed for food web components of various reclaimed systems. Previous studies of benthic invertebrates indicated changes in stable isotope values (^{13}C depletion and ^{15}N enrichment) associated with elevated levels of NAs and PAHs in reclaimed systems. To gain a better understanding of the cycling of carbon and nitrogen sources at the base of the aquatic food web, laboratory studies have been initiated to examine the extent of isotope fractionation associated with the biodegradation of complex mixtures of NAs and PAHs. Tailings pond-derived bacterial cultures were supplied commercial NA mixtures or oil-sands extracted NAs with varying profiles. Microbial growth and changes in CO_2, DIC and DOC concentrations were monitored over time, as were shifts in NA concentration and profile. In addition $\delta^{13}C$ and $\delta^{15}N$ for the biomass, CO_2, DIC, DOC were characterized.

Large volume extraction of naphthenic acids from oil sands tailings pond water and subsequent purification using diethylaminoethyl cellulose. R. Frank[1], R.J. Kavanagh[2], B.K Burnison[3], J.V. Headley[3], K.M. Peru[3], G.J.Van Der Kraak[2] and K.R. Solomon[1]. [1]Department of Environmental Biology, University of Guelph, Guelph, ON; [2]Department of Integrative Biology, University of Guelph, Guelph, ON; and [3]Environment Canada, National Water Research Institute, Burlington, ON.

The oil sands of Alberta's Athabasca Basin contribute to Canada's possession of the second-largest proven oil reserves in the world. The extraction tailings mixture produced during oil sands refining has been reported as being toxic to aquatic organisms and is therefore collected in ponds on site. Investigation into the toxicity of these tailings ponds has identified naphthenic acids (NAs) and their sodium salts as being the major contributors to toxicity. Future toxicity studies require a large volume of a purified naphthenic acid mixture; however a well-defined bulk extraction technique was not available. This study investigated the use of the weak anion exchanger, diethylaminoethyl cellulose, to remove humic-like material from the organic acid fraction of oil sands tailings pond water. The NA extraction and purification procedure proved to be a fast and efficient method to process large volumes of tailings pond water, providing an extraction efficiency of 41.2%. The resulting concentrated NA solution had a composition that differed only slightly from oil sands fresh tailings, with a minor reduction in the abundance of lower molecular weight naphthenic acids being the most significant difference. This reduction was primarily due to the initial acidification of tailings pond water, and the DEAE cellulose treatment appeared to have no significant effect on the NA composition. Ongoing research is attempting to fractionate a NA mixture with the intention of conducting toxicity identification evaluation driven bioassays which will provide a better understanding of the toxicity of individual NAs contained within tailings pond water mixtures.

Microbial carbon sources in wetlands affected by oil sands mining in northern Alberta. C.A. Daly and J.J.H. Ciborowski. Department of Biological Sciences, University of Windsor, Windsor, ON.

Constructed wetlands are a potential landscape remediation strategy following oil sands extraction in northern Alberta. As part of a collaborative effort to understand energy flow in natural and constructed wetlands, I am estimating (i) microbial carbon sources using stable isotope analysis; (ii) microbial production; and (iii) respiration losses (CO_2 and CH_2 evolution) in wetlands of contrasting ages and histories in the Athabasca oil sands region. Amending wetland sediments with peat is a reclamation strategy believed to accelerate wetland succession. These sediments produced six times more CO_2 compared to non-amended wetland sediments. The fresh amendments experienced an almost immediate response in microbial decomposition. However, CH_4 production rates quickly decreased, possibly due to microbes utilizing the labile carbon pools, leaving more recalcitrant compounds to decompose slowly. Low CH_4 production in older highly organic wetland sediments may indicate that methanogenic bacteria are out-competed by sulphur-reducing bacteria. No difference in CO_2 production was found between reference and oil-sands process affected wetland sediments, indicating that microorganisms found both substrate types suitable. Low CO_2 and CH_4 production rates may indicate that newly assimilated organic carbon is efficiently recycled within the wetland food web. If the organic carbon in peat is truly converted into biomass rather than CO_2, then peat amendment appears to be a viable reclamation strategy. It appears that microbial-derived energy in these wetlands may be directed up the food web and not immediately lost to the atmosphere.

Reproductive impairment observed in fathead minnows (*Pimephales promelas*) held in oil sands tailings water. R.J. Kavanagh and G.J. Van Der Kraak. Department of Integrative Biology, University of Guelph, Guelph, ON.

Approximately 35% of the oil that Canada produces comes from the Alberta oil sands. Using the Clarke hot water extraction method, bitumen, a heavy form of crude oil, is separated from oil sand. As a result of this process, large volumes of fluid tailings are produced. Over 3 x 108 m^3 of fluid tailings are currently being stored in settling ponds. Developers plan to transfer these tailings to mined-out pits and cap them with a layer of clean water. An aquatic ecosystem is then expected to be established in these "end pit" lakes. In order to determine if tailings water would have an adverse effect on fish reproduction, a 21 d fathead minnow (*Pimephales promelas*) reproduction assay was conducted at Syncrude Canada. Fathead minnows were held in Gregoire Lake water (reference site) to gather baseline data on fecundity, fertility, and hatching success. Fathead minnows were then held in several types of tailings pond water for 21 d and the same reproductive endpoints were then compared to fish held in Gregoire Lake water. Fathead minnows held in oil sands process water exhibited a cessation of spawning. Female fathead minnows held in this type of tailings water also had significantly reduced gonads and male fish had tubercles that were reduced both in size and number. The high levels of naphthenic acids and salts that are present in tailings pond water are suspected of being responsible for the reproductive impairment observed in fathead minnows. Naphthenic acids appear to inhibit steroidogenesis as goldfish testes stimulated with human chorionic gonadotropin and incubated with naphthenic acid extract (10 $mg \cdot L^{-1}$) produced significantly less testosterone compared to controls. Collectively these studies show that oil sands fluid tailings have the potential to affect the reproductive physiology of fish.

Blowfly infestation of tree swallow (*Tachycineta bicolor*) nests on the Athabasca oil sands toxicity associated with oil sands. M-L. Gentes[1], T. Whitworth[2], C. Waldner[3] and J.E.G. Smits[1]. [1]Department of Veterinary Pathology, University of Saskatchewan, Saskatoon, SK; [2]Whitworth Pest Solutions, Inc., Puyallup, WA; and [3]Department of Large Animal Clinical Sciences, University of Saskatchewan,

Saskatoon, SK.

Oil sands mining companies in Alberta, Canada, are evaluating the feasibility of using wetlands to detoxify process-affected water as a reclamation strategy. Prevalence and intensity of infestation with Protocalliphora sp (Diptera: Calliphoridae) were measured in tree swallows (*Tachycineta bicolor*) on experimental wetlands receiving oil sands mine tailings and compared with those on a reference site. The objectives of this study were to determine if the intensity of parasitism varied with environmental contamination, and if parasite load was associated with the growth and survival of nestlings. Prevalence of infestation on our sites was among the highest ever reported for a small cavity-nester: all 38 nests examined were infested. Mean nest burden on reclaimed wetlands varied from 70.6 puparia per nest (CT wetlands, Suncor) to 76.0 puparia per nest (NW, Suncor), while nests on the reference site harbored an average 44.1 puparia per nest. Nestlings on oil sands reclaimed wetlands suffered individual parasitic load about twice that of nestlings on the control site. Nestling survival was not associated with parasitic load. There was not association between parasitic load and nestling weight on the control site, however, on reclaimed wetlands, heavily parasitized nestlings gained less weight. Factors potentially explaining high blowfly infestation on mining sites included habitat characteristics, the absence of blowfly predators and increased host susceptibility.

Toxicity associated with oil sands process-affected waters and attenuation with time and transport. M.D. Mackinnon[1], P.M. Fedorak[2], W.P. Marsh[2] and A.C. Scott[2]. [1]Syncrude Canada Ltd., Edmonton, AB; and [2]Department of Biological Sciences, University of Alberta, Edmonton, AB.

Associated with development of Alberta's vast oil sands deposits is the production of large volumes of process-affected waters (OSPW). Currently, OSPW is not discharged, but must be contained in geotechnically-secure areas until they are acceptable for release. Processing oil sands alters water properties through addition of dissolved inorganic (salts, trace elements, ammonia) and organic (naphthenic acids (NAs), hydrocarbons) constituents. Fresh OSPW is acutely toxic to aquatic organisms and this must be removed prior to discharge or acceptance of reclamation at oil sands developments. The aquatic toxicity in OSPW is primarily associated with NAs, a group of low molecular weight carboxylic acids. The NAs will impact reclamation strategies of aquatic habitats. The toxicity of OSPW can be mitigated through ageing under aerobic conditions, biological remediation, attenuation during groundwater seepage, or application of selective treatment technologies. Natural ageing processes reduce the NAs, as well as altering the relative composition within the NAs group (higher molecular weight and cyclic character). Recent laboratory studies indicate that biodegradation of the NAs in OSPW will be incomplete, but that toxicity will be reduced to levels that are not detrimental to receiving systems. Reclamation and discharge scenarios must incorporate this so that water management can proceed. In this presentation, some current understandings of the changes in NAs and their toxicity through natural processes will be described.

Tracing aquatic foodweb impacts of oil sands mining process-affected waters using sulphur ($\delta^{34}S$) stable isotopes. M. Elsayeb[1], M.D. MacKinnon[2], D.G. Dixon[1] and M. Power[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Syncrude Canada Ltd., Edmonton, AB.

Syncrude Canada operates oilsands mining leases on the Athabasca oil sands deposits under a zero discharge of process-affected waters commitment. To meet these commitments various aquatic environmental and toxicological studies have been completed. To date few attempts have been made to trace possible waste-water foodweb-related effects or to correlate the severity of observed species-specific effects to trophic position or energy sources. To better understand the cycling of oil sands constituents (polycyclic aromatic hydrocarbons and naphthenic acids) within aquatic environments located on or near the Syncrude lease site, stable sulphur isotope analysis (SIA) was used in

combination with more widely applied carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) SIA. Significant patterns were not evident with the use of carbon and nitrogen SIA, but were evident with sulphur. Sites excavated for experimental work containing varying levels of process-affected water and/or mature fine tailings demonstrated negative S values for all analyzed taxon, while natural or unexcavated sites demonstrated S values around and above 0‰ for most taxon. These results indicate the promise of sulphur SIA for discerning subtle effects of process-affected waters on aquatic foodwebs.

Effects of petroleum coke on the development of aquatic biota in constructed wetlands. L.F. Baker[1], J.J.H. Ciborowski[1] and M.D. MacKinnon[2]. [1]Department of Biological Sciences, University of Windsor, Windsor, ON; and [2]Syncrude Canada Ltd., Edmonton, AB.

Syncrude Canada Ltd. and Suncor Energy Inc. of Fort McMurray, Alberta produce approximately 6 million tonnes of petroleum coke per year as a by-product of oil sands mining. The use of this waste product to stabilize clay-dominated mine tailings in constructed wetlands is currently being studied as an option for landscape reclamation. However, there is limited knowledge of the toxicity of coke and its associated leachates (trace metals and polycyclic aromatic hydrocarbons) to aquatic biota. We studied the in situ effects of petroleum coke on the invertebrate and macrophyte communities of constructed wetlands, and whether or not adding a surface layer of peat would affect community establishment. Treatments consisted of 40 cm diameter x 10 cm thick layers of coke, sand, or natural sediment, with or without a 2 cm thick topping of peat spread on the treatment substrate. Treatments were placed in 3 constructed wetlands in August 2002, June and August of 2003 and 2004. All test patches were collectively sampled at each of these times. Plant cover (%) on each patch was visually estimated. In 2005, macrophyte biomass, species abundance and richness were recorded. Zoobenthos were sampled from colonization tiles that were in place for 8 days. Preliminary results indicate that coke had no effect on macrophyte development. An early, transitory decrease in Chironomidae abundance was observed in coke treatments.

Phototoxicity of oil sands derived polycyclic aromatic hydrocarbons to Japanese medaka (*Oryzias latipes*). A.J. Farwell, V. Nero, M.V. Croft, S.M. Rhodes and D.G. Dixon. Department of Biology, University of Waterloo, Waterloo, ON.

Alkylated polycyclic aromatic hydrocarbons are present in natural and reclaimed aquatic environments in the region of the oil sands in northern Alberta, Canada. An oil sands derived PAH extract, rich in alkylated PAHs, has been shown to induce significant lethal and sublethal responses using the Japanese medaka (*Oryzias latipes*) embryo-larval assay. While information on exposure and effects of oil sands PAHs is available, there is little information on the impact of modifying factors. This study focuses on the effect of solar radiation on oil sands derived PAH extract toxicity. Japanese medaka embryo larval experiments were conducted to determine the toxicity of the photomodified PAH extract following 1 and 4 d exposures to simulated solar radiation (SSR). Next, to evaluate the combined toxic effects of photomodified and photosensitized products of the oil sands PAH extract, Japanese medaka embryos were co-exposed to the PAH extract + SSR, and compared to exposures of a photomodified and non-photomodified PAH extract. Changes in toxicity were evaluated using the following measurement endpoints: mortality, hatch length, and evidence of BSD symptoms. A one day pre-exposure of the PAH extract was sufficient to reduce the toxic effects for all measurement endpoints compared to the non-photomodified extract. A four day pre-exposure of the PAH extract was required to further reduce the effect on hatch length which was the most sensitive endpoint. PAH + SSR (16 h) co-exposure enhanced lethality and the sublethal effects on embryonic development (BSD symptoms) compared to the photomodified (16 h) and non-photomodified PAH exposures. Reductions in hatch length for the PAH + SSR (16 h) co-exposure and the non-photomodified PAH

extract exposure were similar (LOEC, 11 $\mu g \cdot L^{-1}$), whereas the photomodified extract had less of an effect on hatch length (LOEC, 44 $\mu g \cdot L^{-1}$). The 2-fold increase in the toxic effects to embryonic development has implications for environmental risk assessment, monitoring and reclamation strategies.

A novel approach to assess chironomid (Diptera:Chironomidae) community composition in the Athabasca oil sands region. A.M. Swisterski[1], J.J.H. Ciborowski[1], C.M. Wytrykush[1] and C. Beierling[2]. [1]Department of Biological Sciences, University of Windsor, Windsor, ON; and [2]Syncrude Canada Ltd., Edmonton, AB.

My research focuses on using a novel sampling technique, floating exuviae traps, to assess chironomid community composition. Chironomids are an abundant invertebrate family in Alberta's wetlands. They have life-stages in both aquatic and terrestrial environments, making them a useful bioindicator species. The floating exuviae traps are designed to catch emerging Chironomid pupal exuviae (cast-off skins), in a standardized area, to assess community composition. Conventionally, zoobenthic community composition is estimated from dipnet or other benthic sampling methods, and is destructive to substrates and macrophytes. These conventional methods require substantial effort to separate invertebrates from debris. This problem is eliminated by the floating exuviae traps. Traps were sampled every 1, 2, 3 or 6 days over a 12 d period during July 2005. Mean daily emergence estimates suggest that traps can be sampled every 2 d without significant loss of exuviae through sinking or decomposition. Community composition of traditional sampling methods is expected to be comparable to biweekly dipnet samples collected from the floating exuviae traps.

Evaluation of protein expression signatures in the assessment of toxicity of oil sands extracts. S.J. Van Es[1], S.K. Walsh[1], V. Nero[2], A.J. Farwell[2], D.G. Dixon[2] and L.E.J. Lee[1]. [1]Department of Biology, Wilfrid Laurier University, Waterloo, ON; and [2]Department of Biology, University of Waterloo, Waterloo, ON.

The gills and liver in fish are prime targets for toxic actions of many aquatic contaminants. With current high throughput technologies, subtle changes in protein expression due to toxicant action can be readily detected. However, variation in sample preparation, physiological status of individual organisms, genetic variability, etc., can make accurate detection of these changes very difficult. Even more, complexity of techniques, time involvement and costs associated with these procedures make proteomic analysis prohibitive in small labs. We report here a simpler proteomic approach based on the analysis of protein expression signatures (PES) using a trout derived gill and liver cell lines that may be useful for the detection of parallel biomarkers that can be used for evaluation of toxicant action. With mammals, toxicoproteomic technologies using cell lines have proven reliable, repeatable and extremely sensitive. A similar approach using the RTgill-W1 and RTL-W1 cell lines, a trout derived gill and liver cell lines respectively, is reported for the analysis of toxicants derived from oil sands extraction. PES of control and treated cells have been developed using 2D gel electrophoresis and image analysis. Replicate 2D gels of control cells were used to create a "master" gel used to compare against another "master" gel derived from replicate gels of toxicant exposed cells. This proteomic approach allows observations of subtle changes from chemical exposures without the need to identify individual proteins. Distinct differences in protein spots could be used as rapid signature profiling of toxicity.

Regional aquatics monitoring program: addressing aquatic ecosystem health in the Athabasca oil sands region. W.N. Gibbons. Hatfield Consultants Ltd., West Vancouver, BC.

The Athabasca oil sands region is currently experiencing increasing levels of industrial development.

Oil sands projects require large amounts of water for construction and operation, and can potentially influence the aquatic environment. Potential effects range in scale from site-specific to regional level, and may be cumulative over time. The variability in spatial and temporal scales of potential effects, and the multiple potential sources of aquatic stressors, poses a challenge for effective monitoring and protection of the aquatic environment. The Regional Aquatics Monitoring Program (RAMP) is an environmental monitoring program established by a number of oil sands developers to assess changes in rivers, lakes, and aquatic biota as development proceeds. Six components of the aquatic environment (hydrology, water and sediment quality, fisheries, benthic invertebrates, and acid-sensitive lakes) are monitored in an area extending from south of Fort McMurray to the Athabasca River Delta. The objectives of RAMP are to collect baseline data, to monitor the aquatic environment to assess cumulative effects and regional trends, and to integrate findings with traditional ecological knowledge to better understand the aquatic resources of the region. Data collected under RAMP are also used to satisfy regulatory monitoring requirements and to assess environmental impact assessment predictions for oil sands developments. Substantial cost savings to industry and government are realized through coordination of monitoring activities and collaboration with other multi-stakeholder initiatives in the area (e.g., Cumulative Environmental Management Association, Wood Buffalo Environmental Association). Through integration of science-based monitoring and stakeholder cooperation, RAMP provides a basis for the sustainable management of aquatic resources in an ecologically and economically important region of Canada.

Toxicity of oil sands to early life stages of two freshwater fish species. M.V. Colavecchia[1], P.V. Hodson[2] and J.L. Parrott[1]. [1]Environment Canada, National Water Research Institute, Burlington, ON; and [2]Department of Biology, Queen's University, Kingston, ON.

The objectives of this study were to evaluate the influence of natural oil sands on the early life stages (ELS) of fathead minnows (*Pimephales promelas*) and white suckers (*Catostomus commersoni*). ELS sediment toxicity tests were conducted using controls, reference sediments, natural oil sands, and industrially contaminated (wastewater pond) sediments collected from sites along the Athabasca River, Alberta (Canada). Eggs and larvae were observed for mortality, hatching, deformities, growth, histopathological alterations, and cytochrome P-4501A (CYP1A) activity using immunohistochemistry. E-Nat-, S-Nat- and wastewater pond sediment-exposed groups showed significant hatching alterations, reduced growth and exposure-dependent increases in ELS mortality, larval malformations and histopathological indices relative to controls. The most common larval deformities included edemas (pericardial, yolk sac and subepidermal), hemorrhages, and spinal defects. Histological alterations included degenerative, inflammatory and structural alterations in eyes, liver and trunk musculature of exposed larvae. Juveniles white suckers exposed to oil sands and wastewater pond sediments (96 h) demonstrated significantly increased 7-ethoxyresorufin-O-deethylase (EROD) activity (30 to 50 fold) as compared to controls. Reference sediment-exposed groups and water controls demonstrated reliable embryo and larval survival, minimal malformations and negligible CYP1A staining. Sediment analyses using gas chromatography-mass spectrometry revealed high concentrations of alkyl-substituted polyaromatic hydrocarbons (PAH) compared to unsubstituted PAH in natural oil sands (220 to 360 $mg \cdot kg^{-1}$) and oil mining wastewater pond sediments (1300 $mg \cdot kg^{-1}$).These observed signs of blue sac disease (ELS mortality, malformations, growth reductions, CYP1A activity induction) may produce deleterious reproductive effects in natural fish populations exposed to oil sands mixtures.

Municipal waste – pharmaceuticals – detections – effects/Déchets urbains - produits pharmaceutiques-détections-effets

Session co-chair/Président: Karen Kidd

Water and sediment toxicity of selected pharmaceuticals and personal care products to benthic invertebrates. É.B. Dussault[1], V.K. Balakrishnan[2], E. Sverko[3], K.R. Solomon[1] and P.K. Sibley[1]. [1]Department of Environmental Biology, University of Guelph, Guelph, ON; [2]Environment Canada, National Water Research Institute, Burlington, ON; and [3]Environment Canada, National Laboratory of Environmental Testing, Burlington, ON.

Aquatic sediments may represent an important matrix for the deposition and storage of pharmaceuticals and personal care products (PPCPs), yet the potential risks that these compounds pose to sediment-dwelling organisms are virtually unknown. In this study, we evaluated the acute toxicity of the anti-epileptic drug carbamazepine (CBZ), the lipid regulator atorvastatin (ATO), the antimicrobial triclosan (TCS) and the synthetic hormone ethinylestradiol (EE2), toward the midge *Chironomus tentans* and the freshwater shrimp *Hyalella azteca*, using standard 10 d acute toxicity tests. In water-only exposures, LC10 values varied between 0.2 and 28.6 $mg \cdot L^{-1}$ for *C. tentans*, with TCS being the most toxic and CBZ the least toxic compound. *H. azteca* was generally more sensitive than *C. tentans*, with LC10 values ranging from 0.2 to 8.5 $mg \cdot L^{-1}$. The relative toxicity ranking of the four compounds was the same as observed for *C. tentans*; however ATO was approximately 10 times more toxic to *H. azteca* compared to *C. tentans*, and approached the toxicity of TCS. Growth was generally a more sensitive indicator of toxicity than mortality, with EC10 values being 1.5 to 14.7 times lower than LC10 values. Experiments are currently under way to evaluate the acute toxicity of spiked sediments. Measured toxicity thresholds in water-only short-term tests were several orders of magnitude higher than current environmental concentrations, indicating that these compounds likely pose little risk to benthic invertebrates. However, studies to examine chronic responses to PPCPs, particularly effects on reproduction, endocrine disruption and behaviour, are poorly known and will be the subject of future testing.

Disease resistance, stress tolerance and immunological response of *Mytilus edulis* exposed to untreated municipal wastewater. F.M. Akaishi[1], S.D. St. Jean[2], F. Bishay[1], J.D. Clarke[4] and C.A. de Oliveira Ribeiro[1]. [1]Cell Biology Department, Federal University of Parana, Curitiba City, Brazil; [2]Environment Canada, National Water Research Institute, Burlington, ON; [3]Greater Vancouver Regional District, Burnaby, BC; and [4]Environment Canada, Environmental Protection Branch, Dartmouth, NS.

Mussels are being explored as biomonitoring tools for receiving environment monitoring by NWRI and GVRD. As such, the effects of untreated municipal wastewater (UMWW), on blue mussels (*Mytilus edulis*) using the immune system and physiological mechanisms, were investigated to understand mussel response to environmental stress that may be present in wastewater at various levels of treatment. The study was conducted at the New Glasgow, NS treatment plant that services a community of 26 000, and receives primarily a blend of domestic wastewater and stormwater. Fifty mussels per test concentration (5 replicates) were exposed for 21 day (0, 12.5%, 25%, 50% and 100% of UMWW), under static renewal (two renewals of 100% volume per day). Mussels were sampled after 7, 14 and 21 days of exposure for immunological and metabolic assays. On day 21, mussels were also tested for their efficiency to eliminate the bacteria *Vibrio anguillarium*, and integrity of their anaerobic metabolism by exposure to air. Although no significant mortality occurred during the 21 d of exposure, the added stress of a bacterial challenge or continuous air exposure resulted in

higher mortality for mussels pre-exposed to 100% UMWW. Mussels from the control, 25% and 50% groups eliminated most of the bacteria, while 8 mussels from the 50% group and 3 from the control group died after 72 h of continuous air exposure. This suggests a link between mussels exposed to 100% UMWW, and decreased disease resistance and stress tolerance. Immunological assay results were different between UMWW and the control.

Prozac® in the aquatic environment: how can fish be "happy" if they are sexually depressed? V.L. Trudeau, C. Martyniuk, J. Menningen, K.L. Crump, H. Xiong, A. Nadler and X. Xia. Department of Biology, University of Ottawa, Ottawa, ON.

Prozac® (fluoxetine) and the contraceptive steroid ethinyl-estradiol and important contaminants in North American and European waterways. While it is clear that estrogenic chemicals can suppress reproductive function, especially in male fish, less is known about the effects of Prozac and other selective serotonin reuptake inhibitors. Using our brain cDNA microarray (> 9000 cDNAs; see www.auratus.ca) and real-time PCR, we have screened for the effects of injected fluoxetine on the gene expression in female goldfish (*Carassius auratus*) brains. Treatments suppressed plasma estradiol levels 5-fold, Accompanying this physiological response was a 4-fold reduction expression of estrogen receptor (ER) beta in neuroendocrine tissues (telencephalon and hypothalamus. One candidate target for serotoninergic regulation was the neuropeptide isotocin which isotocin is important for stimulating reproductive behaviours in fish. Microarray and polymerase chain reaction analysis has shown that isotocin mRNA levels decreased 6-fold in the brain tissues. It is known that reductions in ERs and in isotocin may help to explain the depressed reproductive activity observed in other studies of fish exposed to the antidepressant Prozac. (Funding source: NSERC).

The effects of gemfibrozil®, a human pharmaceutical found in the environment, change seasonally in goldfish (*Carassius auratus*). T.W. Moon, C. Mimeault, A.J. Woodhouse and V.L. Trudeau. Department of Biology, University of Ottawa, Ottawa, ON.

The human lipid-regulating pharmaceutical gemfibrozil® (GEM) is found in the aquatic environment at concentrations in the $\mu g \cdot L^{-1}$ range. As a fibrate drug, GEM acts as a peroxisomal proliferator (PP) binding to PP-activated receptors (PPARs) that heterodimerize with RXR and ultimately bind to specific DNA-response elements (PPRE). We previously showed that at environmental levels, GEM alters plasma testosterone (T) levels. This study examines the effects of season on changes in T and PPAR gene expression in goldfish (*Carassius auratus*). Goldfish were exposed to environmental doses of GEM through the water for 14 to 28 d at three times during the year. The effects of GEM on T were dependent upon season, but although changes did occur in gonadal PPAR tissue expression, these changes were not always correlated with changes in T. The strong seasonal response to GEM exposure implicates changes in steroidogenesis as a key target of this drug in fish. If correct, both the stress response and reproduction could be adversely affected by GEM. These studies provide further evidence that GEM is an endocrine-disrupting substance and as such may affect the fitness of non-target aquatic species. (Supported by NSERC of Canada Strategic and Discovery Grant Programs).

Assession effects of human and veterinary pharmaceuticals in the environment: approaches and assessment tools. K.R. Solomon. Department of Environmental Biology, University of Guelph, Guelph, ON.

The use of pharmaceuticals is increasing and there is considerable uncertainty regarding pharmaceuticals modes of actions in non-target organisms in the environment. As a class, they present a special case in risk assessment; they are not subjected to routine environmental testing; they are designed to alter physiological responses but not be lethal; they may be pseudopersistent; and they

are usually present as mixtures. Key issues in assessing the potential effects of non-hormonal pharmaceuticals are therefore: potential for interactivity, responses to chronic exposures, and effects other than acute toxicity. In the context of environmental risk assessment, significant effects should be observable in testing procedures that integrate life-cycle functions such as growth, development, and reproduction. These types of responses may be best observed in community-level cosm testing systems that integrate life-cycle tests with opportunity for interactive and indirect effects. Use of aquatic and terrestrial testing systems in which communities are exposed continuously to mixtures of pharmaceuticals in a concentration-response design can allow for the identification of sensitive organisms, key processes in life cycles, and provide margins of exposure between biologically significant effects and environmental concentrations. These approaches, with some examples and some drawbacks will be discussed.

Leukemia incidence in two mussels species, *Mytilus edulis* and *M. trossulus* caged in Burrard Inlet, Vancouver, BC in 2004. S.D. St. Jean[1], F. Bishay[2] and C.L. Reinisch[3]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Greater Vancouver Regional District, Burnaby, BC; and [3]Woods Hole Marine Biological Laboratory, Laboratory of Aquatic Biomedicine, Woods Hole, MA.

Mussels (*Mytilus spp.*) are ubiquitous in Canada and are a vital component of the fisheries industry. These molluscs are continuously screened for pathogens and tumors by Fisheries and Oceans Canada to document the health of both wild and cultured populations. Mussels can also develop leukemia which has been detected by histopathology and more recently by monoclonal antibody (1E10). The Greater Vancouver Regional District is assessing the potential for using mussels as a biomonitoring tool, and in particular the incidence of leukemia, for the receiving environment monitoring program under development for the Lions Gate wastewater treatment plant that discharges into Burrard Inlet, BC. In February 2004, about 1500 mussels for both of the blue (*Mytilus edulis*, from a farmed source) and bay (*M. trossulus*, from wild reference location) mussel species were caged at 5 study sites within Burrard Inlet, and 3 reference sites. Each of the eight study sites had one to three depth stations for a total of 15 depth stations of caged mussels. In addition, four wild populations of *M. trossulus* were also sampled. Sample collection occurred in the spring, summer, and autumn of 2004 and winter of 2005. At each sampling, 20 to 60 mussels of both species per depth station were examined for the presence of leukemia, using both monoclonal antibodies and microscopic evaluation. In addition, 10 mussels of both species were further tested for their phagocytic activity (PA). Results indicate that the bay mussel, *M. trossulus*, was more susceptible to leukemia than the blue mussel, *M. edulis* in 2004
and 2005. Results also indicated at some sites that elevated leukemia induction was weakly correlated to PA; that is, when leukemia is high PA is low. The implication of these results and the utility of leukemia as a bioindicator will be discussed.

CSI: Halifax, Investigating the impact of Halifax Harbour sewage on intertidal species diversity. C. Coray[1], E. Ring[1] and S.M. Bard[2]. [1]Department of Biology, Dalhousie University, Halifax, NS; and [2]Environmental Programmes, Dalhousie University, Halifax, NS.

We established a program (Coastal Scene Investigation: Halifax) to assess species diversity at rocky intertidal beaches to evaluate the impact of Halifax Harbour sewage on coastal habitat. Raw sewage has been flushed into Halifax Harbour from dozens of coastal outfalls for over 250 years. Field sites were established throughout Halifax Harbour along effluent gradients as well as outside of the Harbour at reference sites. Species richness and abundance were assessed at permanent 3.5 x 3.5 m mid- low rocky intertidal quadrat plot using a quadrat study technique to assess both percent of cover

organisms and under-rock species diversity. The baseline data established in 2005 will allow us to monitor the progress of mitigation after a new sewage treatment system is introduced to Halifax Harbour in phases starting in 2006. We also developed a program to experimentally rehabilitate the physical habitat by re-establishing rock cover at a intertidal site in the highly polluted Northwest Arm. We are assessing whether pollution abatement combined with physical rehabilitation of habitat facilitates recruitment of intertidal species to currently depleted sites. The Halifax Harbour sewage situation is unique in Canada in that we had the opportunity to establish an assessment program prior to onset of effluent treatment and plan to monitor the success of remediation on habitat rehabilitation over the coming years.

Effects of municipal wastewater effluents on resident fish populations. G.R. Tetreault[1] M.E. McMaster[2], K. Oakes[1], and M.R. Servos[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Environment Canada, National Water Research Institute, Burlington, ON.

An emerging issue in Canada involves the effects of Municipal Wastewater Effluents (MWWE) in the aquatic receiving environments. MWWE or sewage is a mixture of household waste, ammonia, inorganic chloramines, textile mill effluents (TMEs), and nonylphenol and its ethoxylates and pharmaceuticals all of which have been detected in environmental samples. The Grand River Watershed receives the outflow of 26 sewage treatment plants, in addition to runoff from other sources. At low flow periods, it is estimated that the Grand River below Brantford is a high percent treated sewage. With this study, we are interested in determining whether MWWE effects normal reproductive development in fish and if so, does impaired reproductive health influence the survival of fish populations, and alter the fish community? In order to address the issue of endocrine disruption, we conducted reproductive evaluations in selected sentinel fish species (*in vitro* sex steroids, and gonadosomatic indices). As well we assessed fish responses in terms growth, reproduction and survival, upstream and downstream of the Kitchener and Brantford sewage treatment plants where existing NWRI studies are monitoring pharmaceutical levels. Studies downstream of the Kitchener plant revealed Johnny darters (*Etheostoma nigrum*) to be of smaller stature and have lower liver somatic index than fish collected at the reference site. However, the capacity of follicular tissue from these fish to produce sex steroids was more responsive to forskolin stimulation. Female common shiners (*Luxilus cornutus*) collected downstream of Kitchener effluent discharge also had an increased capacity to produce testosterone and 17β-estradiol. Similar to the Kitchener study, collections downstream of the Brantford MWWE resulted in smaller mature Johnny darters and a trend for follicular tissue to have greater capacity to produces steroids. Bluntnose minnows (*Pimephales notatus*) from the same site had smaller livers when compared to reference fish collections, and demonstrated alterations in sex steroid production. Although this is preliminary data, project is currently in its second year, and progress regarding the impact of MWWE on fish assemblages will be discussed.

Effects of pharmaceuticals on a human hepatocellular carcinoma (HepG2) bioassay. R. Karaga, N. Tytka and P.F. Dehn. Department of Biology, Canisius College, Buffalo, NY.

Pharmaceuticals have been discovered worldwide in sewage effluents, surface water, and ground water. The purpose of this study was to examine cytotoxicity and biotransformation responses of a human bioassay, exposed to low-levels (0.001 to 10 mM) of carbamazepine, clotrimazole, phenobarbital, and propranolol for 96 h. Cytotoxicity (neutral red assay) and biotransformation responses (cytochrome P450 1A1/2 (EROD) and 2B (PROD) activities) were assessed. Clotrimazole was the most cytotoxic (70% viable as compared to solvent controls at 0.01 mM) followed by propranolol (16% at 0.25 mM), carbamazepine (87% at 0.75 mM), and phenobarbital (23% at 10

mM). Carbamazepine at 0.001 mM significantly increased (115%) cell viability above solvent controls. EROD activity was significantly increased by all, however propranolol increased activity at the lowest level (112% at 0.05 mM) followed by clotrimazole (205% at 0.1 mM), carbamazepine (154% at 2.5 mM), and phenobarbital (241% at 10mM). PROD activity was significantly increased by all; however, propranolol, clotrimazole and propranolol increased activity at the lowest level (737% and 183%, respectively at 0.1 mM), followed by carbamazepine (159% at 0.75 mM) and phenobarbital (272% at 10mM). Clotrimazole alters membrane permeability, thus a cytotoxic response at low concentrations was not unexpected. Carbamazepine, propranolol and phenobarbital are known substrates for human CYP2C8 and 3A4, 2C19 and 2D6, and 2C9 and 2C19, respectively, yet only phenobarbital and carbamazepine have been shown to induce CYP2B6 and 3A4 or 3A4, respectively. Our data indicates that all pharmaceuticals tested can induce CYP1A1/2 and 2B, which are common bioindicators of exposure in field/epidemiological studies.

Impact of non-steroidogenic anti-inflammatory drugs (NSAIDs) on heat-shock protein (HSP) response in rainbow trout (*Oncorhynchus mykiss*) larvae. A. Gravel and M.M. Vijayan. Department of Biology, University of Waterloo, Waterloo, ON.

The objective of this study was to investigate whether non-steroidogenic anti-inflammatory drugs (NSAIDs) are playing a role in the HSP response of early life stages of fish. In the first part of the study, rainbow trout (*Oncorhynchus mykiss*) larvae were exposed to different concentrations (0.1, 1, 10, 100, 1000 $\mu g \cdot L^{-1}$) of salicylic acid (SA) or ibuprofen in water for 96 h. Analysis by Western blot demonstrated a significant induction in liver hsp70 levels with 10 to 1000 $\mu g \cdot L^{-1}$ ibuprofen compared to the control group. In the second part of the study, larvae were exposed to two different concentrations (1 and 1000 $\mu g \cdot L^{-1}$) of either SA or ibuprofen in the water and compared to a control group. After 96 h, fish were subjected to a +10 °C heat shock for 1 h and then allowed to recover, while samples were taken at 0, 1, 4 or 24 h post-heat shock. Results demonstrated that liver hsp70 expression increased significantly at 4 and 24 h after a heat shock in rainbow trout larvae. However, the hsp70 response to heat shock was very rapid with a high dose of ibuprofen (1000 $\mu g \cdot L^{-1}$). The values reached a maximum immediately after a 1 h heat shock and the decrease in hsp70 levels post-heat shock was not as rapid as the other groups. Considered together, our results suggest that NSAIDs alter the cellular heat shock response in rainbow trout larvae.

Acidic pharmaceuticals in surface waters of selected Ontario watersheds. D.T. Bennie[1] and J. Struger[2]. [1]Environment Canada, National Water Research Institute, Burlington, ON; and [2]Environment Canada, Ecosystem Health Division, Burlington, ON.

Pharmaceutical products are used to control human diseases and conditions as well as enhance animal health and increase food production efficiency. These substances enter the environment via sewage treatment plant (STP) effluent discharges, land application of sewage sludges and agricultural animal wastes. This study set out to determine the temporal occurrence of acidic pharmaceuticals in surface waters at 6 sites in Hamilton Harbour (HH) and 6 sites in the Grand River in southern Ontario. Both watersheds receive multiple inputs from STPs. Monthly samples were collected from these locations from May 2003 to May 2004 and were analyzed for 12 acidic pharmaceuticals. Preliminary data shows four pharmaceuticals were detected regularly at levels above analytical detection limits. Those substances were ibuprofen, gemfibrozil, naproxen and triclosan. The maximum concentrations found in the study were 1590, 103, 753 and 428 $ng \cdot L^{-1}$, respectively. Other detectable substances include clofibric acid, salicylic acid, diclofenac, indomethacin and fenofibrate. In HH, concentrations of pharmaceuticals decrease significantly in the gradient from the STPs to the Harbour to Lake Ontario. Distribution patterns on the Grand River are different due to the increased number

of STP discharge contributions at periodic intervals to the River.

Additive and non-additive interactions of binary xenoestrogen mixtures in zebrafish (*Danio rerio*). L.L. Lin[1] and D.M. Janz[2]. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; and [2]Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, SK.

This study evaluated effects of binary mixtures of two common xenoestrogens on sex ratios, vitellogenin (Vtg) induction, heat shock protein 70 (hsp70) expression, gonadal cell death, and breeding success in zebrafish (*Danio rerio*). Fish were exposed from 2 to 60 days post – hatch (dph) to environmentally relevant concentrations of 17α-ethinylestradiol (EE2) (1 or 10 $ng \cdot L^{-1}$ nominal; EE1 or EE10), 4–nonylphenol (NP) (10 or 100 $\mu g \cdot L^{-1}$ nominal; NP10 or NP100), binary combinations of EE2 and NP (EE1+NP10, EE1+NP100, EE10+NP10, or EE10+NP100) or solvent control (acetone; 0.1% v/v) in a static-renewal system with replacement every 48 h. At 60 dph, 40 fish from each treatment were euthanized for analysis, while the remainder were raised in clean water for reproductive studies. Significant increases in weight-at-length of treatments EE10, NP100, EE1+NP10, EE10+NP10 and EE10+NP100 were observed when compared to the control. Delayed gametogenesis in males and females in EE10, EE10+NP10 and EE10+NP100 were observed, while no testicular tissues were detected in groups EE1, EE1+NP10 and EE1+NP100. Significant decreases in total number of eggs collected from treatments EE10 and EE10+NP100 were observed when compared to solvent controls. No eggs were produced from EE10+NP10. Hatchability of eggs was significantly lower in EE10 when compared to the control. Assessment of potential correlation between hsp70 expression and gonadal cell death will be performed. Overall, our data suggest the effects of EE2 and NP are additive up to a threshold level of EE10+NP10, while at EE10+NP100, NP appeared to antagonize the action of EE2.

Environmental impacts of freshwater aquaculture/Impacts environmennementaux de l'aquiculture d'eau douce

Session co-chairs/Présidents: Karen Kidd and Cheryl Podemski

Environmental studies of some cage fish aquaculture operations in Ontario. M.N. Charlton and J.E. Milne. Environment Canada, National Water Research Institute, Burlington, ON.

Freshwater fish cage aquaculture is a relatively new industry in Ontario. Fifteen working farms, mainly in the Georgian Bay/Manitoulin Island area of Lake Huron, produce from 10,000 lb to over 50,000 lbs of harvest annually. Because there were few data available on environmental effects, we have made a preliminary study of conditions at three working farms and at one discontinued site. Each site was visited winter and summer between 2000 and 2005. Water quality surveys were conducted in receiving waters near each farm. Generally, nutrient concentrations were close to background levels near the farms. The "footprint" of faeces deposition under farm operations was determined by mapping the organic content of cores. Most deposition falls directly beneath the cages. Preliminary results of a following study show a decrease in fish deposition after a 6 month following period at farm in Lake Woolsey. With the use of an underwater camera and a "penetrometer" the thickness of fecal deposits has been observed at a discontinued site every year between 2000 and 2005. Results indicate complete reduction of the deposit would occur over 10 years by 2010. Shoreline algal populations thought to indicate time integrated nutrient effects were surveyed for evidence of attached algae near 6 caged aquaculture sites in July 2003 and June 2005. No direct influence of fish farms was evident.

Are metal deposits below a caged fish aquaculture operation detrimental? *Hyalella azteca* toxicity tests. W.P. Norwood, M.N. Charlton and J.E. Milne. Environment Canada, National Water Research Institute, Burlington, ON.

Sediments collected from inside the "footprint" of feces deposition under a discontinued farm operation were assessed by a number of techniques. First, Cu and Zn sediment concentrations were evaluated in relation to sediment quality guidelines. Second, concentrations of Cu and Zn released from the sediments into the overlay water of chronic toxicity tests were compared to Water Quality Guidelines as well as to Critical Water Concentrations (LC25's) determined for the aquatic invertebrate *Hyalella azteca*. Finally, preliminary chronic toxicity tests with *H. azteca* indicate that there was little impact on survival. The biological results will be discussed in relation to sediment and water quality guidelines and the importance of using a weight-of-evidence approach in the interpretation of assessment data.

Effect of fish farms on contaminant levels in wild biota. A. deBruyn[1], N. Eyding[1], H. McNally[2], J. Harding[3], R. Mountain[4], C. Marlor[5], D. Urban[6], S. Verenitch[1] and A. Mazumder[1]. [1]Department of Biology, University of Victoria, Victoria, BC; [2]Ahousaht First Nation, Ahousaht, BC; [3]Kitasoo/Xaixais First Nations, Klemtu, BC; [4]Musgamaqw Tsawataineuk Trial Council, Alert Bay, BC; [5]Department of Sociology, Rutgers University, Piscataway, NJ; and [6]BC Aboriginal Fisheries Commission, West Vancouver, BC.

An emerging issue in salmon aquaculture is the fate and effects of toxic chemicals, including intentionally-used products and persistent organic pollutants that are present in feed. Several of these contaminants have been identified in farmed fish flesh and more recently in sediments near farms. We have now extended this assessment to wild biota living down-current of farms. With the assistance of local harvesters in each of three partner First Nations' territories in coastal British Columbia, we sampled clams, prawns and demersal fish from sites near fish farms and from reference sites. Levels of PCBs, organochlorine pesticides and heavy metals in these species were highly variable among regions, among sites and among species, but indicated some significant differences between near-farm and reference sites. Stable isotope analysis revealed that some of these changes may be attributable to changes in the trophic position of rockfish near some farms. In other cases, we conclude that there are changes in contaminant patterns at the base of the food web near farms. Contaminant concentrations in long-lived species such as rockfishes change over a longer time scale than cycles of production and fallowing, and thus at least some impacts of fish farms may not be considered transitory.

The Experimental Lakes Area aquaculture project: overview of the first two years. C.L. Podemski[1], K.H. Mills[1], D. Findlay[1], M.J. Turner[1], C. Bristow[2], P.J. Blanchfield[1], M.J. Patterson[1], L. Tate[1], R.C. Rooney[3], M.A. Kullman[4] and K.A. Kidd[4]. [1]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; [2]Department of Biology, University of Ottawa, Ottawa, ON; [3]Department of Entomology, University of Manitoba, Winnipeg, MB; and [4]Department of Biology, University of New Brunswick, St. John, NB.

There is increasing pressure from concerned nongovernmental organizations and regulatory agencies to improve our scientific understanding of the environmental costs of aquaculture. Although substantial research has occurred in the marine environment, relatively little attention has been focussed on freshwater ecosystems. Researchers at the Experimental Lakes Area (northwestern Ontario) are conducting a whole-lake experiment examining the mechanisms through which cage aquaculture impacts lake ecosystems. A 10 tonne per year all-female rainbow trout (*Oncorhynchus mykiss*) cage was installed in Lake 375 in 2003. Impacts from the cage operation on water quality,

primary productivity, phytoplankton, zooplankton, and changes in sediment characteristics and benthic invertebrate communities are being monitored. Trap-netting in spring and fall is used to document the abundance and condition of forage fish and the population size, condition, and recruitment of lake trout (*Salvelinus namaycush*) and white suckers (*Catostomus commersoni*). Depth-sensing acoustic telemetry is being used to monitor the impacts of the cage farm on the spatial distribution of native lake trout and white sucker. The presentation will provide the audience with an overview of environmental impacts observed during the first two years of farm operation.

Assimilation of freshwater aquaculture waste into a native food web. M.A. Kullman[1], K.A. Kidd[1] and C.L. Podemski[2]. [1]Department of Biology, University of New Brunswick, St. John, NB; and [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

A whole ecosystem study at the Experimental Lakes Area was initiated in 2002 to examine the effects of freshwater aquaculture on lake ecosystems. The experimental aquaculture operation cultured 10,000 rainbow trout (*Oncorhynchus mykiss*) yearly over the summers of 2003 to 2005, following current industry practices. The feed used in the industry and in the experiment is comprised primarily of marine herring, resulting in a feed with isotopic signatures that are distinct from energy sources within a freshwater ecosystem. Stable isotope ratios of native biota were compared before and after the introduction of aquaculture. After one season of aquaculture, there were no changes in the stable isotope signatures of littoral invertebrates, zooplankton, Mesiad, or fish from the background data, indicating that the feces and excess feed provided by the operation did not become an important dietary component. After two years of cage culture, a shift in the $\delta^{15}N$ in zooplankton (3.6-4.9‰ in 2002 to 5.1-8.7‰ in 2004) and Mesiad (3.9-4.6‰ in 2002 to 5.7-6.8‰ in 2004) towards the signature of the feed (7.1‰) suggests that they are using the wastes provided by the aquaculture operation as a novel energy resource.

The effects of freshwater aquaculture on lake sediments and benthic invertebrate community structure. E.C. Rooney[1] and C.L. Podemski[2]. [1]Department of Entomology, University of Manitoba, Winnipeg, MB; and [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

Freshwater aquaculture is a potential area for diversification in the agricultural sector, but regulators have been reluctant to permit the development of new farms or the expansion of existing ones. This is largely due to the lack of scientific understanding regarding the industry's environmental impact. We examined the effect of aquaculture waste on sediments and associated benthic invertebrates at an experimental rainbow trout (*Oncorhynchus mykiss*) farm, located in Lake 375 at the Experimental Lakes Area (north-western Ontario). Unionized ammonia concentrations in pore-water of the sediment situated beneath the farm exceeded 30 $\mu g \cdot L^{-1}$. The concentrations of Zn and Cu in WP were also elevated within 3 m of the cage's edge, reaching 1335 $mg \cdot kg^{-1}$ and 200 $mg \cdot kg^{-1}$ respectively. These and other chemical effects were sharply delineated and were not detectable beyond 5 m of the cage's edge. Over the study period, the dominance of Ostracoda in sediments beneath the farm declined while Chironomidae increased. Within the Chironomidae, members of the *Tanytarsini* were numerically dominant, however, the proportion of chironomids belonging to the Chironomini increased. There was a sharp decline in invertebrate density within 10 m of the cage's edge. At the end of the facility's second season in operation, the region within 3 m of the cage's edge was afaunal. The effect of the farm on the invertebrate community was detectable over a larger area than indicated by chemical analyses alone, implying that sediment chemistry monitoring is not an adequate surrogate for invertebrate sampling.

Estimation of waste outputs by a rainbow trout (*Oncorhynchus mykiss*) cage farm using a nutrient mass-balance approach. P.A. Azevedo[1], D.P.Bureau[2] and C.L. Podemski[1]. [1]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB: and [2]Department of Animal and Poultry Science, University of Guelph, Guelph, ON.

Assessments of environmental impacts from cage culture operations are often done by direct monitoring of their nitrogen (N) and phosphorus (P) waste outputs. This is costly, yet inaccurate. Studies have indicated that a mass(nutrient) balance approach of estimating waste outputs provide more robust estimates than direct monitoring of waste outputs (Cho 1994, Bureau et al. 2003). Total solid wastes, solid P and N, and dissolved P and N wastes from cage farmed rainbow trout were estimated over two production cycles at the Experimental Lakes Area Research Station, Kenora, ON using the bioenergetics feed requirement and waste output model (Fish-PrFEQ, Cho and Bureau 1998, Bureau et al. 2003). Chemical analysis of samples of the feed (average composition: crude protein, 44%; lipids, 22% and P, 1.04%, as is basis), fish through the two production cycles, and fecal material, coupled with information on feed inputs and growth were used as model inputs or coefficients. In 2003, water temperature averaged 15 °C. The fish grew from 90 g to 900 g with a thermal-unit growth coefficient (TGC, growth rate) of 0.195, and a feed conversion ratio (FCR = dry feed / live weight gain) of 1.13. Total solid, solid N and P and dissolved N and P waste outputs were estimated as 267, 13.6, 6.1, 39.3, and 2.6 kg per tonne of fish produced, respectively. In 2004, the fish grew from 100 to 1000 g with a TGC of 0.242 and FCR of 1.09 at 14 °C. Total solid, solid N and P and dissolved N and P waste outputs were 236, 11.3, 5.6, 37.6, and 2.4 kg per tonne of fish produced, respectively. Estimates of waste outputs from the model correlated well with N and P concentrations in water samples collected during the two production cycles.

Management and prediction of waste impacts from freshwater aquaculture and connection to the science-policy interface. R.D. Moccia[1], G. Reid[1], D.P. Bureau[2], M. Glinka[1] and F. Salazar[1]. [1]Aquaculture Centre, University of Guelph, Guelph, ON; and [2]Department of Animal and Poultry Science, University of Guelph, Guelph, ON.

Sustainability of the Canadian freshwater aquaculture industry will require, in part, improved tools to predict, manage and regulate the water quality effects resulting from cage aquaculture. Some of these effects are due to the accumulation and dispersal of solid, organic fecal material as well as the production of other soluble nutrients such as phosphorus. Consequent effects on near-field P concentrations in the epilimnetic waters, as well as additive Biochemical Oxygen Demand (BOD) to the hypolimnion, are two of the major factors limiting the long-term environmental sustainability of fish farms in the Great Lakes. This research aims to develop tools to help predict near-field P concentrations and hyplolimnetic BOD impacts resulting from cage aquaculture facilities in the Great Lakes. Stable isotope signatures are being identified to help track the dispersal and relative contributions of farm-derived waste to the benthos, and to distinguish these from other sources. BOD evaluation of fish feces produced from various feed formulas is also underway, and will support the production of an environmental impact model to aid in the prediction of hypolimnetic effects. Improvements to the assessment of near-field P concentrations are also being made, by coupling waste output information with a simple dispersion and dilution model using current velocity and direction information. Finally, we aim to incorporate these biological and water quality indicators into a conceptual Decision Support Tool (DST), which may be used to improve the regulatory management of freshwater fish farms.

Aquatic ecotoxicity - phytotoxicology/ Écotoxicité aquatique - phytotoxicologie

Session chair/Président: Beverley Hale

Characterizing the herbicidal effects of statin pharmaceuticals in *Lemna gibba*. R.A. Brain[1], T.S. Reitsma[1], J.K. Beestari[1], L. Lissemore[2] and K.R. Solomon[1]. [1]Department of Environmental Biology, University of Guelph, Guelph, ON; and [2]Ontario Ministry of Agriculture, Food and Rural Affairs, Laboratory Services Division, Guelph, ON.

Statins are synthetic lipid-lowering agents which competitively inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) in the treatment of high cholesterol. HMG-CoA is one of the most highly regulated enzymes in mammals as well as plants. In plant systems HMG-CoA is the rate determining step in the cytosolicmevalonic acid pathway where isopentenyldiphosphate (IPP) is synthesized. IPP provides the basic building block of the terpenoid biosynthetic pathway. An autonomous pathway in the chloroplast has also been characterized which synthesizes IPP, however, although there is some crosstalk between pathways, the anabolic products are considered largely distinct. The mevalonic acid pathway is responsible for the synthesis of sterols and the side-chain of ubiquinone, which are critical for membrane fluidity and electron transport, respectively. The plastidic pathway is responsible for the synthesis of carotenoids, chlorophylls, and plastoquinone, which are critical components of the photosynthetic pathway. Statin pharmaceuticals have previously been considered not to have significant impacts on anabolic plant processes through the inhibition of HMG-CoA, however our laboratory studies with *Lemna gibba* indicate that statins do cause concentration-dependent toxicity via reduction of mevalonate (HMG-CoA mediated) derived products. This presentation will discuss the concentration-dependent effects of atorvastatin and lovastatin on the levels of plastoquinone, ubiquinone, β-sitosterol, and stigmasterol in *L. gibba* quantified using HPLC-UV/Vis. Extraction and quantification protocols developed for quinones and sterols as well as atorvastatin and lovastatin will be detailed.

Toxicity of mass occurrences of pelagic cnidarians in enclosed coastal ecosystems. B.D. Johnston[1], I. Sötje[2], V.R. Dayeh[3], N.C. Bols[3], G. Alexander[4], C.J. Secombes[1] and U. Båmstedt[5]. [1]College of Life Sciences, University of Aberdeen, Aberdeen, United Kingdom; [2]Department of Zoologie, University of Hamburg, Hamburg, Germany; [3]Department of Biology, University of Waterloo, Waterloo, ON; [4]University of Oslo, Oslo, Norway; and [5]Umeå Marine Sciences Centre, Umeå, Sweden.

Widespread mortalities of fish in fjords and sea lochs in Norway the United Kingdom, as well as increasing reports human exposure to toxic jellyfish in the Mediterranean and elsewhere have prompted concern that mass occurrences of gelatinous zooplankton may be as important as harmful algal blooms as sources of toxicity in certain marine environments. The toxic effects of several pelagic cnidarians including, *Cyanea capillata*, *Aurelia aurita*, *Periphylla periphylla*, and *Muggeia atlantica* were investigated. Comparative studies on the behavioural, physiological, and molecular effects of exposure to cnidarian toxin in teleost and mammalian models were performed. Toxic effects included hemolysis, cardiotoxicity, inflammation, and cellular necrosis. Densities of gelatinous zooplankton were determined and related to toxic equivalencies in the water column by estimates of total molecular concentrations of toxins in the blooms of the pelagic cnidarians. The conclusions drawn by these studies indicate that mass occurrences of gelatinous zooplankton may have significant toxicological consequences in enclosed ecosystems affecting human activity and potentially causing shifts in community structure leading to the competitive exclusion of teleosts.

Mortalities of caged Atlantic salmon (*Salmo salar*) and paralytic shellfish (PS) toxins in zooplankton, shellfish and crustaceans associated with a bloom of *Alexandrium fundyense* off Grand Manan, New Brunswick, Canada. D.W. Sephton[1], K. Haya[2], J.L. Martin[2], M.M. LeGresley[2] and F.H. Page[2]. [1]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS; and [2]Department of Fisheries and Oceans, St. Andrews Biological Station, St. Andrews, NB.

In September 2003, mortalities of Atlantic salmon (*Salmo salar*) at two aquaculture sites in Long Island Sound, off Grand Manan, NB, Canada were attributed to a harmful algal bloom (HAB) of > 3 X 105 cells·L^{-1} of *Alexandrium fundyense*, a dinoflagellate which produces a group of potent neurotoxins that cause paralytic shellfish poisoning (PSP) in human and vertebrate consumers. *A. fundyense* had PS toxin contents of 19.5 pmol STX eq (saxitoxin equivalents) $cell^{-1}$ and a PS toxin profile rich in high-potency carbamates (78.5%). The profile found in zooplankton was similar. Salmon collected at death had low levels of PS toxins (< 4 μg STX eq·100 g^{-1} wet weight) in gut, gill and muscle tissue. This suggests that intoxication and death occurs rapidly, prior to the accumulation of unsafe levels of PS toxins (regulatory limit (RL) of 80 μg STX eq·100 g^{-1}) in edible flesh.

Blue mussels (*Mytilus edulis*) growing on the salmon cages had very high levels of PS toxins (37,500 μg STX eq·100 g^{-1}) and their PS toxin profiles had a greater proportion of carbamates than that found in *A. fundyense*. Blue mussels collected from an adjacent Canadian Food Inspection Agency (CFIA) monitoring site had PS toxin contents of 4210 μg STX eq·100 g^{-1} and 150 μg STX eq·100 g^{-1} in late September and December respectively. The December level was still well above RL, indicating that a period longer than 13 weeks was necessary for the PS toxin contents of mussels to decline to levels safe for harvesting following this HAB event. Horse mussels, *Modiolus modiolis*, collected in late September had PS toxin contents of 2630 μg STX eq·100 g^{-1}. Laboratory detoxification experiments indicated that contaminated blue mussels may have required up to 20 weeks to reach RL, a function of the high PS toxin levels accumulated.

Lobsters (*Homarus americanus*) from Long Island Sound had high PS toxin contents in hepatopancreas (447 μg STX eq·100 g^{-1}), and stomach (85 μg STX eq·100 g^{-1}) tissues, but were below RL in muscle and gill. Lobsters from nearby Duck Island Sound and Flagg Cove had PS toxin levels above RL (177 μg STX eq·100 g^{-1} and 243 μg STX eq·100 g^{-1}, respectively) in hepatopancreatic tissue only, while lobsters from Whale Cove had PS toxins contents below 40 μg STX eq·100 g^{-1} in all tissues. The PS toxin profile of hepatopancreas from Long Island Sound lobsters was dominated by gonyautoxins and/or C-toxins. These data illustrate the movement of PS toxins through the marine food chain (algae – zooplankton – bivalves and crustacean scavengers) following a HAB event. While PS toxin levels are monitored in suspension feeding shellfish to ensure the safety of human consumers, the potential for PS toxicity resulting from consumption of contaminated lobster hepatopancreas and gonadal tissue should not be overlooked.

The use of transgenic canola (*Brassica napus*) and plant growth-promoting bacteria for the phytoremediation of a nickel contaminated site. A.J. Farwell[1], S.Vesely[1], H. Rodriguez[2], V. Nero[1], D.G. Dixon[1] and B.R. Glick[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Cuban Research Institute on Sugarcane By–Products, Havana, Cuba.

Laboratory studies have indicated the potential for use of canola (*Brassicanapus L.*, cv. Westar) with novel traits and the plant growth-promoting bacterium (PGPB) (*Pseudomonas putida* UW4) as a method to remediate Ni-contaminated soil. Transgenic canola expressing the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase under the transcriptional control of the rolD promoter and the bacterium *P. putida* UW4 were tested for the ability to remediate Ni-contaminated soil in the field. This enzyme activity can degrade ACC that is produced by plant roots in response

to a variety of stresses, including the presence of Ni, and thereby increase plant growth and provide greater quantities of above ground biomass for harvest in remediation efforts. To determine the efficacy of transgenic canola and/or PGPB as a method for Ni remediation, field trials were conducted in southern Ontario at a site contaminated with elevated levels of Ni (6500 $mg \cdot kg^{-1}$). In the first field trial (June 9 to July 13, 2004) non-transgenic canola and transgenic canola with two copies of the ACC deaminase gene under the control of rolDpromoter produced similar shoot biomass compared to multi-copy and single copy transgenic canola with the rolD promoter. The non-transgenic and double copy transgenic canola showed similar changes in shoot biomass (< 1.0 to 8.0 g) with increasing Ni content in the shoots (< 80 $mg \cdot kg^{-1}$ shoot dry weight). As a result of partial flooding, the evaluation of *P. putida* UW4 for plant growth promotion in the second field trial (August 31 to October 5, 2004) was limited to plants from low Ni contaminated soil (<28 $mg \cdot kg^{-1}$ shoot dry weight). The available data showed significant increases in growth for both non-transgenic and double copy transgenic canola inoculated with *P. putida* UW4. Additional field trials (2005) will be conducted to further evaluate the use of transgenic canola and/or PGPB in metal remediation.

Lethality of microalgae to farmed Atlantic salmon (*Salmo salar*). L.E. Burridge, J.L. Martin, M.C. Lyons, M.M. LeGresley and B.D. Chang. Department of Fisheries and Oceans, St. Andrews Biological Station, St. Andrews, NB.

Blooms of phytoplankton in the Bay of Fundy have been implicated in the deaths of farmed Atlantic salmon (*Salmo salar*). The purpose of this study was to establish whether or not elevated concentrations of these algae can kill salmon. Monocultures of two species of microalgae, *Alexandrium fundyense* and *Ditylum brightwellii* were grown in large quantities and Atlantic salmon smolts were exposed to a range of concentrations of these cultures for 24 h. The LC50 was determined and reported according to the concentration of cells present ($cells \cdot L^{-1}$). Fish exposed to *D. brightwellii* at concentrations as high as 10^6 $cells \cdot L^{-1}$ had no apparent deleterious effect. This concentration is well above the concentration observed in the field. Some salmon exposed to *A. fundyense* died during the exposure period. The LC50 was estimated to be 642,000 $cells \cdot L^{-1}$, a concentration that has been observed in the field. *A. fundyense* is a know neurotoxin producer and is the organism responsible for causing paralytic shellfish poisoning. Work is continuing to determine if other species found in the Bay of Fundy can cause problems for cultured fish and to determine the concentration of toxins in the *Alexandrium* cultures and fish tissues.

Metal uptake by vegetables and herbs grown in lightstation gardens in British Columbia. H. Damman[1], N. Healey[1], C. LaCoste[2] and B. Ibbotson[3]. [1]Department of Fisheries and Oceans, Institute of Ocean Sciences, Sidney, BC; [2]Health Canada, BC; and [3]Water and Earth Science Associates Ltd., Toronto, ON.

An understanding of how plant type and plant part affect the uptake of metals from soils would allow us to assess the exposure to contaminants by various kinds of herbivores, including humans. Residents on the lightstations along British Columbia's coast often garden on soils contaminated with various metals. Analysis of paired plant-tissue and soil samples collected from lightstation gardens revealed several patterns in metal uptake: (i) metal content of edible plant parts generally increased as metal concentration in the soil increased; (ii) leaf- and shoot-based crops generally had a higher metal concentration than either root or fruit crops grown on similarly contaminated soils; and (iii) some contaminants were taken up more readily than others. Three models of soil-contaminant uptake by plants were compared to evaluate which approach allows us to most reliably predict the human-health risks associated with eating produce grown on contaminated soils.

Aquatic ecotoxicity – methods development/Écotoxicité aquatique - développement de méthodes

Session chair/Président: Patti Gillis

pH Stabilization procedures during the testing of acute lethality of municipal wastewater to rainbow trout (*Oncorhynchus mykiss*). L.J. Novak[1], L.N. Taylor[2] and R.P. Scroggins[2]. [1]Stantec Consulting Ltd., Guelph, ON; and [2]Environment Canada, Environmental Technology Centre, Ottawa, ON.

Ammonia is a pH dependant toxicant in municipal wastewater effluent. The Environment Canada rainbow trout (*Oncorhynchus mykiss*) test method (EPS 1/RM/13) is commonly used to determine acute lethality of effluents. As required in this test method, aeration of wastewaters may cause the pH to rise due to equilibration of CO_2 partial pressure in the effluent with that in the atmosphere. Such a rise in pH may in turn affect ammonia speciation, thereby shifting ammonia from the less toxic ionized species to the more toxic un-ionized or free form. If sufficient residual ammonia is present in a final treated municipal wastewater sample, the resulting increase in acute lethality could be the result of aeration required by the test method (termed "artifactual toxicity") or just a normal rise in wastewater pH over time. To address this concern, Environment Canada is preparing a testing document which allows the use of three pH stabilization procedures (i.e., direct CO_2 injection, CO_2 recycling or pH controller) to control pH drift during rainbow trout acute lethality testing with municipal wastewater effluent. These procedures will reduce the potential for artifactual ammonia toxicity. However, prior to using a pH stabilization procedure, municipalities must obtain approval by government regulatory authorities. Approval for use will be considered only when five specific criteria have been met.

The influence of water chemistry on the uptake and toxicity of cadmium in *Chironomus riparius*. P.L. Gillis and C.M. Wood. Department of Biology, McMaster University, Hamilton, ON.

The Biotic Ligand Model (BLM) is a mechanistically based tool which can be used to predict site-specific metal toxicity based on water chemistry. The majority of data used to develop the BLM were derived from trout, fathead minnow (*Pimephales promelas*) and *Daphnia* toxicity tests. In an effort to extend the BLM to a wider range of species, we investigated the effect of varying water chemistry on the sensitivity of midge larvae (*Chironomus riparius*) to waterborne Cd. Standardized toxicity tests with *C. riparius* use 2nd instar larvae and to sustain the animals during the test, both food and substrate are added. Because organic matter (food) can complex dissolved metals, we chose to avoid feeding during the test and therefore employed 3rd and 4th instar larvae, since they were much less susceptible to starvation during the exposure (48 h). Time course uptake experiments using Cd^{109} demonstrated that larvae accumulate Cd for the duration of the exposure. Initial range-finder Cd exposures were conducted in both moderately-hard water (Ca 1000 μM, Na 600 μM, Mg 200 μM, pH 7.9, DOC 3 $mg \cdot L^{-1}$), and soft water (Ca 50 μM, Na 50 μM, Mg 20 μM, pH 7.2, DOC 0.7 $mg \cdot L^{-1}$). The 48 h LC50 in soft water was 260 $mg \cdot L^{-1}$ versus 1100 $mg \cdot L^{-1}$ in hard water. The concentration of Cd in the tissues of exposed chironomids reflected the high exposures, reaching 3 $mg \cdot g^{-1}$ in soft water exposures and 40 $mg \cdot g^{-1}$ in hard water. Even in soft water, the larvae were still extremely tolerant to Cd, withstanding concentrations in the millimolar range. At these levels, even extreme changes in water chemistry will result in only minor effects on Cd sensitivity. Therefore, in order to extend the BLM to *C. riparius* environmentally realistic Cd concentrations need to be investigated. Ongoing studies will address the sensitivity of earlier life stages and the potential of using these stages in full BLM-type experiments. (Supported by CDA, ICA, ILZRO, INCO, NiPERA,

Noranda-Falconbridge, Teck Cominco and an NSERC CRD grant).

Crayfish as a biomonitor for point source contaminant characterization. G.R. Craig[1], I. Middelraad[2] and C. Portt[3]. [1]G.R.Craig & Associates, Schomberg, ON; [2]D.G. Dixon & Associates, Guelph, ON; and [3]C. Portt & Associates, Guelph, ON.

Crayfish (*Cambarus robustus*) collected from the Speed River upstream, downstream and adjacent a ditch discharge, known to transport PCBs, accumulated concentrations that differed between sites separated as little as 10 m. Crayfish contain 1.5 to 3% lipid which is in the lower range for indigenous fish species (1 to 7%) and higher than two mussel species (about 1%) used in this biomonitoring study. PCB congener accumulation in crayfish and mussels show a predominate similarity with the exception of a few congeners. Accumulation of PCBs was greater among the larger crayfish but lower concentrations in smaller crayfish may represent shorter exposure. An optimum size of crayfish to best reflect a single season of exposure has been determined from this data set. Ease of collection, adequate lipid reserves, confined territoriality, wide geographic availability, its role as a benthic consumer and food source for riparian birds and mammals makes crayfish an excellent organism for organic contaminant monitoring and risk assessment of predators.

It's time to abandon co-incidence sediment quality benchmarks. D.W. Smith and S.M. Jones. Conestoga Rovers & Associates, Inc., Exton, PA.

In the 1980s, regulatory agencies in Canada and the United States were faced with a dilemma. Contamination of aquatic sediments was simultaneously serious, up to 43 percent of U.S. sediments are supposedly contaminated enough to be a danger to human health and the environment (EPA 2004), and very expensive to remediate. Adding to this already combustible mix, the science at the time was insufficiently developed to address the complexity of sediment chemistry and potential toxicity. Given this quandary, a wide range of risk assessors and regulatory agencies resorted to several types of co-occurrence sediment quality benchmarks (SQBs). or example, there are the effects range-low (ERL) and effects range-median (ERM) benchmarks produced by NOAA scientists (Long and Morgan 1991), the lowest effects level (LEL) and severe effects level (SEL) produced by Ontario Ministry of the Environment (Persaud et al. 1994), and the threshold effects concentration (TEL)/probable effects concentration (PEL) (Smith et al. 1996). The information contained in these SQBs was then used to developed so-called consensus SQBs, the threshold effect concentration (TEC) and probable effect concentrations (PEC) (Macdonald et al. 2000).

While the exact methods of derivation vary, the different co-occurrence SQBs share basic similarities. All contain two tiers of SQB values. Lower tier SQBs (ERL, TEC, TEL, and LEL) identify sediment concentrations for individual chemicals below which impacts to aquatic life are unlikely. Upper tier SQBs (ERM, SEL, PEC, and PEL) are concentrations of a chemical above which impacts on aquatic life are, ostensibly, likely. Another commonality among the different co-occurrence SQBs is that they are all based on the co-incidence of the sediment concentration of a specific chemical with some impact to benthic organisms, either reduced diversity or toxicity observed in bioassays.

A critical factor is that the sediments used in the derivation of SQBs are taken from the field, including anthropogenically-impacted areas near large cities. Use of field-collected sediments, as opposed to spiked-sediments, is simultaneously seen as both a key advantage and a critical failing of these benchmarks. On the plus side, the resulting information on toxicity or impact is based on the potential effects at the concentrations and the combinations of chemicals that actually occur in the field. On the other hand, the presence of multiple potential causal factors makes it all but impossible to identify the chemical, or chemicals, that are causing toxicity. The potential for spurious

relationships between toxicity and any one toxic chemical is especially problematic for aquatic sediments. In general, large urban areas typically have multiple sources of many toxic chemicals. Moreover, sediment toxicants tend to accumulate in the same fine organic-rich sediments. High concentrations of toxic metals tend to co-occur with high concentrations of other metals, other toxic agents (e.g., PAH, pesticides), and other stressors (e.g., ammonia, hydrogen sulfide, and low dissolved oxygen (O'Connor 1999a and b, Peddicord and Lee 1998). Consequently, it is widely acknowledged that SQBs cannot demonstrate causality between toxicity and any specific chemical, although this caveat is as widely ignored when applying these SQBs.

Given the ongoing controversy surrounding the co-occurrence SQB, we took a close look at how they are actually derived to better understand what they are actually defining. As a starting point, we began with the ERL and ERM values (Long and Morgan 1990). These SQBs are derived as follows. A suite of sediments is assembled spanning a range of concentrations and from a range of locations, including areas impacted by anthropogenic discharges. Sediments are classified as impacted or not impacted, and the impacted sediments are sorted according to the concentrations of a toxic chemical. The ERL value is defined as the 10th percentile concentration of impacted sediments, and the ERM is the 50th percentile concentration for that chemical. It is important to note that this process is generally repeated for multiple chemicals using the same group of sediments. That is, concentrations of different chemicals from the same sediment sample are often used to generate SQBs for multiple chemicals.

To better understand the phenomena that are actually considered when deriving an SQB, one must understand the relationship between chemical concentrations and "impacts" to biota. Fig. 1, adapted from data presented in Ingersoll et al. (2002), depicts a typical relationship between sediment toxicity and chemical concentrations. As depicted, the percentage of bioassays that are toxic has an S-shaped dose response to increasing chemical concentrations. At very low chemical concentrations, the percentage of sediment samples that are toxic is low and unresponsive to increasing concentrations. At moderate chemical concentrations, toxic sediments become increasingly more common as concentrations rise. At very high chemical concentrations, the prevalence of "toxicity" eventually levels off at the maximum, 100 percent of bioassays.

While the shape of this dose response is typical, the reader should note that initial part of the dose response is not centered on zero percent. For these and other bioassays, there is a baseline, or background toxicity, that ranges from about 10 to 20 percent of tests, depending on the bioassay (Ingersol et al. 2002). Because ERLs are the 10th percentile concentration of impacted sediments, inspection of Fig. 1 suggests that ERLs will normally be located in this area of background toxicity. Consequently understanding this background "toxicity" is critical to understanding ERLs.

What causes this background toxicity? Some portion of this background toxicity is Type I statistical error. Environmental analyses typically employ a significance level of 0.05 to identify when a sample is toxic. Consequently, about one in 20 tests, or 5 percent of bioassays, is expected to exhibit significant "toxicity" simply due to chance. Another likely cause of this background "toxicity" is potentially non-toxic factors, such as grain size and sediment type (O'Connor 2004), which can also significantly affect the growth and survival of benthic macroinvertebrates. A third factor is potentially biased controls. Laboratories conducting bioassays have strong disincentives associated with poor survival and growth of control treatments. Bioassays that are rejected because of unacceptable control performance must be repeated. Hence, testing laboratories will likely, over time, tend to select control sediments that are optimal for growth and survival of control organisms. There may also be biological factors (e.g., parasites and predators) that are likely to be more problematic in sediments from unknown locations compared to favorite control sediments, in which survival and growth are routinely monitored. There is also potentially bias in interpretation of results. Poor survival in experimental

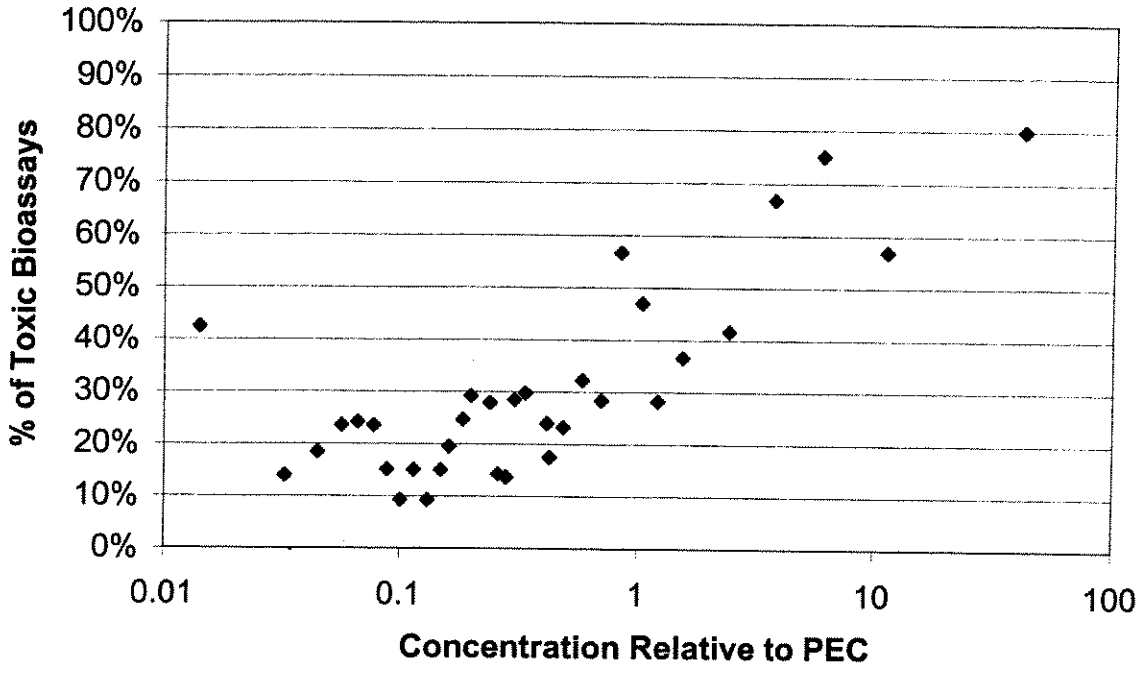

Fig. 1. Sediment toxicity vs. chemical concentration

sediments is interpreted as toxicity. Poor survival in control sediments is interpreted as a failed test that needs to be redone. It is important for the reader to note, and note again, that none of the causes of background toxicity described above are actually ecotoxicological effects.

Actual toxicity also contributes to the background toxicity. However, the flat dose response (Fig. 1) suggests that even the actual toxicity component of this background toxicity is due to chemicals other than the target chemical. These other chemicals will include naturally occurring toxic metabolites (ammonia and sulfide), as well as other toxic anthropogenic chemicals. Last and likely least, in terms of contribution to background toxicity, is toxicity due to the target chemical.

Simultaneous inspection of the methods used to derive an ERL value and the dose-response of sediment toxicity to a potentially toxic chemical suggests an important, but strange, phenomenon. The ERLs are widely used de facto toxicological endpoints, but they are probably based largely, or perhaps even entirely, on phenomena other than toxicity. If any actual toxicity contributes to the derivation of an ERL, most and potentially all of that toxicity is due to other chemicals. By simple logic, then, the toxicity of the target compound has little, potentially nothing, to do with the final magnitude of its ERL.

If the value of a lower tier SQB is not based on the toxicity of that chemical, then what is it based on? Because the lower tier SQBs are actually based on the spurious background toxicity, we hypothesized that these SQBs must be based on background concentrations. To test this hypothesis, we used linear regression to compare the ERL and the other lower tier co-occurrence SQBs (TEC, TEL, LEL) for metals to the median background concentrations for aquatic sediments reported by Rice (1999). As can be seen from Fig. 2, median background concentrations for these metals (As, Cd,

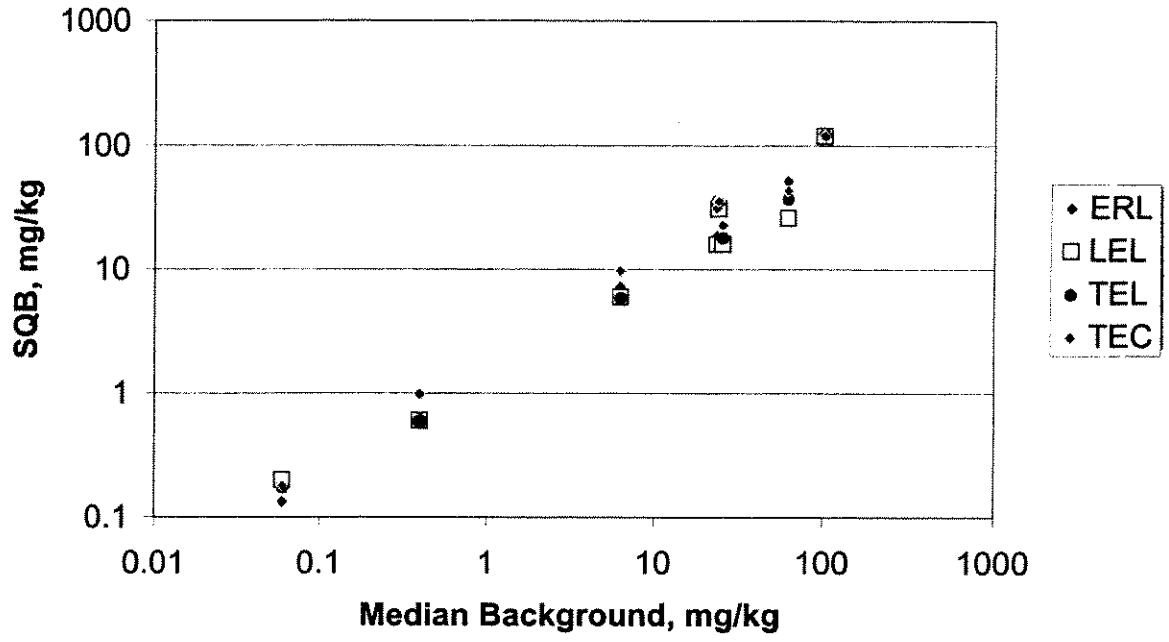

Fig. 2. Median background concentrations of metals vs. lower tier SQB

Cr, Cu, Pb, Hg, Ni, and Zn) are very robust predictors of the lower tier SQBs. All of the regressions yield r^2 values of 98 percent or greater, and the relationship to median background levels is close to isolinear (i.e., the slope is about equal to one). As opposed to actual toxicological benchmarks, lower tier SQBs are apparently simply indicators of about median background concentrations.

Armed with this knowledge, we then investigated the upper tier SQBs. Keeping in mind that the ERM is the 50th percentile of impacted sediments, we hypothesized that the upper tier SQBs should be located in the middle of the S-curve depicted in Fig. 1. Observed toxicity in this part of the curve is likely due to the spurious background toxicity and actual toxicity. The positive dose response in this region suggests that the actual toxicity is due to the target chemical and/or toxicity to other chemicals whose concentrations correlate those of the target chemical.

Put more formally, we hypothesized that the magnitude of an upper tier SQB should be a function of the background concentration of a chemical and it's toxicity in sediments. We tested this hypothesis in two ways. First, the difference between the upper tier SQBs and their median background concentrations were compared to four indices of toxicity and exposure in sediments. The estimators of toxicity/exposure were: (i) the U.S. EPA ambient water quality criteria; (ii) median sediment- water partitioning coefficients (Kd) (EPA 1999), (iii) an estimated safe sediment concentration based on equilibrium partitioning and the two previous estimators; and (iv) the Netherlands' maximum permissible concentration (MPCs) for sediments (Crommentuijnn et al 1997). The MPC values are safe-sediment concentrations based on Netherlands water quality criteria and observed Kd levels. The testable hypothesis was that the more toxic metals (e.g., Cd) would have upper tier SQBs closer to background concentrations than less toxic metals (e.g., Zn). Secondly, we conducted stepwise multiple regression of the upper tier SQBs by entering, as independent variables, these four indices of sediment

toxicity/exposure along with three different estimators of background concentrations. The estimators of background were median concentrations for non-urban sites, median concentrations for urban sites, and the 75th percentile for all sediment samples, also from Rice (1999). To normalize the residuals, the natural logs (ln) of all variables were used. The second method allows the statistical analysis to partition the effect of background and toxicity on the final value of an SQB.

The results of the first test are presented in Table 1. Contrary to our expectation, none of the four estimators of toxicity/exposure for the metals correlated significantly and positively with the difference between "ln SQB" and "ln background". In several cases, the relationship was significant but contrary to our hypothesis. A similar result was obtained with the stepwise regression. Only indices of background concentrations entered into the stepwise regression as significant predictors of the upper tier SQBs. Median metals concentrations for urban sites were the best predictors of SEL, PEC, and PEL values, whereas the 75th percentile concentrations were the best predictor of ERM values. Even though only one independent variable entered into the stepwise regression, the r^2 values for the four regressions still ranged from 93 to 97 percent. In short, no index of toxicity was a significant predictor of any of the upper tier SQB, even when the contribution of background was removed manually by subtraction or statistically by multiple regression. Contrary to our hypothesis and widespread belief, the relative magnitudes of upper tier SQB are not significantly related to the relative toxicity of the metals considered. Only background concentrations are significant predictors of the magnitude of upper tier SQBs. Like their siblings, upper tier SQBs are also apparently simply indicators of background concentrations, albeit a higher percentile of background.

Table 1. Results of correlation analyses of toxicity/exposure parameters vs. difference between median background concentrations (BG) and upper tier SQBs. "Ln" is natural log, "WQC" refers to U.S. water quality criteria, "Kd" is the sediment concentration estimated with those two factors, and "MPC" is the Maximum Permissible Concentration. Statistically significant ($p < 0.05$) correlations are bolded. Note the predicted relationship was between these parameters was positive.

	Ln WQC	Kd	Ln (WQC, Kd)	Ln MPC
Ln ERM - Ln BG	-0.59	-0.24	-0.21	**-0.74**
Ln SEL - Ln BG	**-0.79**	0.02	-0.39	**-0.69**
Ln PEC - Ln BG	**-0.77**	-0.08	-0.48	**-0.77**
Ln PEL - Ln BG	**-0.77**	-0.05	-0.46	**-0.74**

If true, then the magnitude of an SQB will be based the prevalence of a metal in the earth's crust and in sediments, not the metal's toxicity. To test this prediction, we focused on Hg, the rarest of the metals which has the lowest SQBs. The lower and upper tier SQBs for Hg average about 0.15 mg·kg

^{1} and 1.1 mg·kg^{-1}, respectively. Given the conventional and likely incorrect belief that SQBs are indicators of toxicity, the low Hg SQBs are often mistaken as evidence for very high toxicity of Hg. However, high Hg toxicity to benthic organisms is not consistent with either theory or empirical data. Safe sediment concentrations predicted from safe water concentrations and equilibrium partitioning theory range from 26 mg·kg^{-1} (Crommentuijnn et al 1997) to as high as 700 mg·kg^{-1} (this study). A well-controlled, chronic bioassay with spiked-sediments showed no effects on midge growth or survival at 1000 mg·kg^{-1} Hg (Thompson et al. 2002). In the field, sediments with Hg concentrations ranging from 10 to 70 mg·kg^{-1} pose no apparent impact to benthos (e.g., see Reynoldson 1998, Milani et al. 2003). In fact, we could find no compelling evidence that Hg is toxic in aquatic sediments, even at concentrations orders of magnitude above the SQBs.

The co-occurrence SQBs are widely used as de facto toxicological standards for sediment contamination. However, our analysis demonstrates that they have no clear relationship to the toxicity. Instead of toxicity, lower tier SQBs are apparently simply indicators of about median background concentrations, while upper tier SQBs are indicators of some upper percentile of background concentrations. For obvious reasons, the acceptable uses and utility of background values are fundamentally different than for toxicological values. Therefore, in our opinion, use of these SQBs as de facto toxicological standards is not warranted and should be discontinued. It should be remembered that co-occurrence SQBs were developed as stop-gap measures until more scientifically robust methods could be developed. Several of these methods have recently been proposed (e.g., Crommentuijn et al 1997, EPA 2005). We recommend that these methods, which have a toxicological basis, replace the use of co-occurrence SQBs.

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Aquatic ecotoxicity – CPY1A/Écotoxicité aquatique - Cyp 1A

Session chair/Président: Peter Hodson

Does contamination of fish eggs with PCB126 affect the response of larvae to diazinon. C.M. Couillard, M. Lebeuf, L. Benoît and S. Trottier. Department of Fisheries and Oceans, Institut Maurice Lamontange, Mont-Joli, Qc.

During their formation, fish eggs may receive a load of persistent organic compounds from their mother and then, after spawning, be exposed to pesticides present in water. The effect of treatment of eggs with 3,3',4,4',5 pentachlorobiphenyl (PCB 126) on the responses of larvae to the organophosphorous pesticide diazinon, was evaluated in mummichog (*Fundulus heteroclitus*). A few hours after fertilisation, eggs were treated topically with a solution of PCB 126 in dimethyl sulfoxide (DMSO) (Group P), DMSO (Group D) or not treated (Group N). Newly-hatched larvae from Groups P and D were exposed to diazinon in saltwater (125 to 12,900 $ng \cdot L^{-1}$) and Group N larvae to saltwater alone in a 96 h static test with daily renewal of the solutions. In Group D larvae, diazinon caused an inhibition of cholinesterase (ChE) activity at concentrations $\geq$ 361 $ng \cdot L^{-1}$ and a reduction in body length at 12,900 $ng \cdot L^{-1}$. Both responses were proportional to the concentration at $\geq$ 361 $ng \cdot L^{-1}$. Treatment of the eggs with PCB 126 caused a 8-fold induction of the activity of ethoxyresorufin-O-deethylase (EROD) in larvae and altered their responses to diazinon. It increased the ChE activity by 10% but it reduced body length by 1%. This improvement in ChE activity without associated improvement in body length indicates that the reduction in body length observed in larvae exposed to diazinon is not entirely related to ChE inhibition. Further studies are needed to assess the overall impact of the observed interaction between PCB 126 and diazinon on growth and survival of fish larvae.

Alpha-naphthoflavone is both a synergist and antagonist of retene toxicity to larval fish. J.A. Scott. Department of Biology, Queen's University, Kingston, ON.

Embryos and larvae of fish exposed to alkyl-substituted PAH such as retene (7-isopropyl-1-methylphenanthrene) develop blue sac disease (BSD). This is a syndrome characterized by edema,

haemorrhaging, deformities, and induction of cytochrome P450 (CYP1A) enzymes when PAH bind to the arylhydrocarbon receptor (AhR) protein. To determine if retene's toxicity is due to parent retene, or to a metabolite following retene oxygenation by CYP1A enzymes, larval trout were co-exposed to graded concentrations of retene and to alpha-naphthoflavone (ANF). ANF also causes CYP1A induction but is a potent antagonist of CYP1A enzyme activity. If parent retene is the toxic form, inhibition should antagonize toxicity. ANF by itself was non-toxic at the concentrations tested. However, at low exposure concentrations, it potentiated retene toxicity with dramatic increases in the rates of BSD and mortality. HPLC analysis of tissue and bile demonstrated increased numbers and concentrations of dihydroxy metabolites, but decreased numbers and concentrations of monohydroxy derivatives. At high concentrations of ANF, the toxicity of retene was completely eliminated, suggesting that the toxic form was a mono-oxygenated derivative. This was confirmed by tissue analyses demonstrating that the parent retene predominated when toxicity was absent. This may be the first reported case of both potentiation and antagonism by the same compound. It also demonstrates the difficulty of predicting the toxicity of complex mixtures of hydrocarbons that have different interactions with the AhR and CYP1A proteins.

Metabolism and toxicity of alkyl-anthracenes in rainbow trout (*Oncorhynchus mykiss*). D. Turcotte[1], N. Abudulai[1], P.V. Hodson[2] and R.S. Brown[1]. [1]Queen's University Department of Chemistry, Queen's University, Kingston, ON; and [2]Department of Biology, Queen's University, Kingston, ON.

Fish are often exposed to polycyclic aromatic hydrocarbons and the toxicity and metabolism of those toxicants is still not completely understood. The excretion mechanism of xenobiotics is composed of a two-step metabolic process. The first step (phase I), which renders compounds more polar, involves cytochrome P450 (CYP) enzymes. The second step (phase II) involves the conjugation of phase I intermediates with glucuronic acid, glutathione, sulfate or amino acid moieties. CYP enzymes, although part of a mechanism of detoxification, often enhance the general toxicity of anthracene (ANT) derivatives by forming reactive oxygen species. The purpose of this study was to investigate the metabolism of ANT and alkyl-ANTs by rainbow trout (*Oncorhynchus mykiss*). Phase I metabolites were investigated *in vitro* by incubating single ANT and alkyl-ANTs with postmitochondrial supernatant fraction (S9 fraction). Phase II metabolites were investigated *in vivo* by exposing rainbow trout to the same compounds through intraperitoneal injection for a period of 24 h, after which the gall bladder was sampled. Phase I and biliary phase II metabolites were identified using HPLC coupled with a UV-diode array absorbance detector. Alkyl-chain and ring hydroxylated compounds were found as phase I intermediates. Alkyl-chain and ring glucuronide conjugates were also found for all alkyl-ANTs investigated. Ring conjugates only were found for ANT, as expected. Further metabolized glucuronide conjugates were also detected which are suspected to be diglucuronide conjugates.

Identification of compounds in crude oil that cause CYP1A induction in fish. C.W. Khan[1], G. Saravanabhavan[2], R.S. Brown[2] and P.V. Hodson[1]. [1]Department of Biology, Queen's University, Kingston, ON; and [2]Department of Chemistry, Queen's University, Kingston, ON.

Crude oil is a major source of polycyclic aromatic hydrocarbons (PAHs) in the aquatic environment. Individual PAHs cause developmental malformations in the early life stages of fish and we have observed similar effects in larval fish exposed to crude oil in the laboratory. Induction of cytochrome P450 (CYP1A) enzymes is characteristic of developmental toxicity caused by crude oil, and increased CYP1A enzyme activity is an effective biomarker of PAH uptake. Using a Toxicity Identification and Evaluation (TIE) approach with different crude oils, we found that CYP1A induction in trout varied

widely after exposure to four unique crude oil fractions (F1 – white gas, F2 – kerosene, F3 – coal tar/bitumen, F4 – wax). F3, which contained the highest concentration of PAHs, accounted for most of the CYP1A induction caused by whole oil. F4 (containing high molecular weight PAHs) also caused moderate CYP1A induction, while F1 and F2, which contained primarily two ring PAHs, caused none. Our current research is testing CYP1A inducing potential of sub-fractions of F3 generated by cold acetone extraction. Cold acetone extraction at –20 °C, –40 °C, and –80 °C separated lower molecular weight compounds (65% of total carbon) from wax (35% of total carbon); all CYP1A induction was associated with the acetone extract. In the future, we will fractionate the extract further with normal-phase HPLC followed by reverse-phase HPLC. CYP1A-active fractions will be analyzed chemically to identify which specific PAHs are associated with these fractions.

Crude oil fraction toxicity to early life stage Japanese medaka (*Oryzias latipes*). L.M. Clarke and P.V. Hodson. Department of Biology, Queen's University, Kingston, ON.

Crude oil is a complex mixture with each crude being unique in chemical composition and PAH concentration. The presence and concentration of polycyclic aromatic hydrocarbons (PAHs) has been linked to the dioxin-like toxicity in early life stages of fish exposed to crude oil. This toxicity has been characterized by the presence and severity of blue-sac disease and the induction of cytochrome P4501A (CYP1A) enzymes. Two crude oils, Scotian Shelf (Scot) and Alaska North Slope Crude (ANSC), were fractionated into 4 fractions (F1 to F4), which were then used in chronic early life stage Japanese medaka (*Oryzias latipes*) toxicity experiments. Embryos were exposed to a chemically enhanced water accommodated fraction (CEWAF) of one of the fractions. The F3 fraction, which contains many PAHs and alkyl PAHs, was found to have the highest toxicity in both oils. F1 and F2 had high mortality which was attributed primarily to narcotic effects as toxicity scores remained relatively low. ANSC F4 had a relatively low toxicity in terms of both sublethal and lethal effects.

In vitro aquatic toxicology/Marqueurs de toxicité in vitro

Session chairs/Présidents: Kristin Schirmer and/et Vivian Dayeh

Applications and potential uses of RTgill-W1, a trout derived gill cell line, in aquatic toxicology. L.E.J. Lee[1], V.R. Dayeh[2] and N.C. Bols[2]. [1]Department of Biology, Wilfrid Laurier University, Waterloo, ON; and [2]Department of Biology, University of Waterloo, Waterloo, ON.

Gills are unique structures to aquatic organisms and the availability of the trout derived gill cell line, RTgill-W1, is beginning to make impacts in fish health and toxicology. These cells are available from the American Type Culture Collection as ATCC CRL 2523. The cells have an epithelioid morphology and form tight monolayer sheets that can be used for testing epithelial resistance. The cells can be grown in regular tissue culture surfaces or in transwell membranes in direct contact with water on their apical surfaces. The ability of RTgill-W1 to withstand hypo- and hyper-osmotic conditions and their optimal growth capacity at room temperature, make these cells ideal sentinel models for *in vitro* aquatic toxicology. The cells have been used to understand mechanisms of toxicity, ranking the potencies of toxicants, and evaluating the toxicity of environmental samples. The cells are also valuable for high throughput toxicogenomic and toxicoproteomic studies which are easier to achieve with cell lines than with whole organisms. RTgill-W1 cell line could become a valuable complement to whole animal studies and in some cases as gill replacements in aquatic toxicology.

An *in vitro* fish cell bioassay for detecting ammonia in industrial effluent. V.R. Dayeh[1], K. Schirmer[2] and N.C. Bols[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and

[2]Department of Cell Toxicology, UFZ – Center for Environmental Research, Leipzig, Germany.

Effluent samples from a nitrogen product producer were used to establish practical protocols for screening effluent with bioassays that utilize fish cell lines. As most fish cell lines grow indefinitely, such bioassays reduce the use of fish in toxicity testing, and in turn, the costs of testing. In addition, the time necessary for cellular responses to develop can be relatively shorter, which means results are obtained more quickly. Methodology was developed for presenting the effluent to the cells without extraction. This was achieved by adding to the effluent the amounts of salts, galactose and sodium pyruvate, as solids, that were necessary to give concentrations and osmolality of the basal growth medium, L-15. Measuring cell viability was compared with three fluorescent indicator dyes: alamar Blue™, CFDA-AM, and neutral red. A few of the effluents tested caused vacuolization, which could be quantified with neutral red. The effluents contained ammonia and further studies on the toxicity of ammonia to the rainbow trout cell lines were conducted. The results of the effluent cytotoxicity were compared favorably with the rainbow trout lethality bioassay. Vacuolization by fish cells in culture is a promising response for identifying effluents that are toxic due to ammonia.

Hepatocyte cultures as an alternative to live fish in environmental estrogenicity studies. J.P. Sherry, T. Hooey and L.M. Hewitt. Environment Canada, National Water Research Institute, Burlington, ON.

Laboratory bioassays for the measurement of estrogenic responses in rainbow trout (*Oncorhynchus mykiss*) are most often based on the induction of the egg yolk precursor vitellogenin (Vg) in immature or male fish. Those bioassays usually last 21 d, consume reasonably large numbers of fish, and require a wet laboratory or its equivalent. Use of *in vitro* alternatives can overcome those problems. We are using an *in vitro* test that is based on the induced production of vitellogenin (Vg) in primary cultures of rainbow trout hepatocytes as an alternative to live fish in environmental estrogenicity studies. We shall describe some of the key performance characteristic of the test. We shall also describe some experiments to assess the effect of dosing route on the tests performance. Our test now includes a cytotoxicity endpoint to provide a useful measure of a test substance's upper boundary concentration. We shall present results from our use of the in vitro test to assess the estrogenicity of pulp and paper mill effluents and their components.

Ah receptor activation and stress response: insights from trout corticosteroidogenesis. N. Aluru and M.M. Vijayan. Department of Biology, University of Waterloo, Waterloo, ON.

Anthropogenic stressors activating aryl hydrocarbon (Ah) receptor signaling, including polychlorinated biphenyls, impair the adaptive corticosteroid response to stress, but the mechanisms involved are far from clear. Using the Ah receptor agonist β-naphthoflavone (BNF) and the antagonist resveratrol (RVT), we tested the hypothesis that steroidogenic pathway is a target for endocrine disruption by xenobiotics activating Ah receptor signaling. Trout (*Oncorhynchus mykiss*) were fed BNF (10 $mg \cdot kg^{-1}d^{-1}$), RVT (20 $mg \cdot kg^{-1}d^{-1}$) or a combination of both (RBNF) for 5 d, and subjected to a handling stress. BNF induced cytochrome P4501A1 (CYP1A1) expression in the interrenal tissue and liver, while this response was abolished by RVT, confirming Ah receptor activation. In control fish, handling stress transiently elevated plasma cortisol and glucose levels and transcript levels of interrenal steroidogenic acute regulatory protein (StAR), cytochrome P450 cholesterol side chain cleavage ($P450_{scc}$) and 11β-hydroxylase over a 24 h period. BNF treatment attenuated this stress-induced plasma and interrenal responses; these BNF-mediated responses were reverted back to the control levels in the presence of RVT. We further examined whether these *in vivo* impact of BNF on steroidogenesis can be mimicked *in vitro* using interrenal tissue preparations. BNF depressed ACTH-mediated cortisol production and this decrease corresponded with lower StAR and $P450_{scc}$, but not

11β-hydroxylase mRNA abundance. RVT eliminated this BNF-mediated depression of interrenal corticosteroidogenesis *in vitro*. Altogether, xenobiotics activating Ah receptor signaling are corticosteroid disruptors, and the mode of action includes inhibition of StAR and $P450_{scc}$, the rate limiting steps in steroidogenesis.

Potential mechanisms for the breakdown of pulp and paper-derived plant sterols to endocrine-disrupting compounds. M.R. Van Den Heuvel[1], F.D.L. Leusch[2] and B. McKague[3]. [1]Department of Biology, University of Prince Edward Island, Charlottetown, PE; [2]CRC Water Quality and Treatment, Brisbane, Australia; and [3]Pulp and Paper Centre, University of Toronto, Toronto, ON.

The causative agents of reproductive impacts of pulp and paper effluents remain unknown. Androgenic and estrogenic effects related to effluent exposure have both been observed. Though plant sterols have been implicated, in the case of androgenic or estrogenic impacts this is unlikely because plant sterols do not act as by either mechanism. The purpose of this study is to examine the hypothesis that oxidation products of sterols contribute to androgenic or estrogenic activity in effluents. Sterols were oxidized using chlorine dioxide in order to mimic the chlorine bleaching process. The main chemical products were found to be oxidized sterols. Receptor binding bioassays, aromatase inhibition, and mosquitofish (*Gambusia affinis*) masculinisation studies were conducted in order to examine endocrine-disrupting potential. Neither the parent sterols, nor the oxidation products showed the ability to masculinize mosquitofish. Androgen receptor binding activity was observed in both the parent sterols and in the oxidations products. Estrogen receptor binding activity was negligible. Further fractionation experiments were conducted in order to determine the nature of the active compounds. Activity was not associated with the sterols as receptor-binding corresponded to the known retention window of steroids. Further GC-MS analysis tentatively identified 5 different steroids; many of those steroids are androstane structures known to have the ability to bind the androgen receptor. This study concluded that the chlorine dioxide oxidation of sterols is unlikely to be a mechanism for the production of androgenic or estrogenic compounds. However, the contamination of even reportedly pure sterols with androgenic compounds is of concern for toxicological studies.

Use of the ciliated protozoan *Tetrahymena* as a test organism in aquatic toxicology. M.D.O. Pinheiro[1], D. Sotornik[1], V.R. Dayeh[1], M.E. Power[1], B.J. Butler[1], R. Slawson[2], L.E.J. Lee[2], D.H. Lynn[3] and N.C. Bols[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Department of Biology, Wilfrid Laurier University, Waterloo, ON; and [3]Department of Integrative Biology, University of Guelph, Guelph, ON.

The free-living ciliates, *Tetrahymena pyrifrormis* and *T. thermophila*, are easily grown in the laboratory, making them in the past valuable experimental organisms in several disciplines, including pharmacology, but they also are important industrially, as part of the sewage treatment process, and ecologically, as predators in microbial food chains. Lately, they have begun to be used in ecotoxicology. However, their use in toxicology disciplines would be improved by the development of methods for exposing and monitoring multiple cultures. To this end we have developed assays for measuring *Tetrahymena* viability, proliferation, and phagocytosis that are done in multiwell plates and measured with a multiwell plate reader. Under development are assays to monitor the impact of toxicants on the bacterivory of *Tetrahymena*. The utility of these simple assays is being explored in studies on environmental pharmaceuticals, sewage treatment process, oil sands wastewater, and mining effluent. Among five metals commonly found in mining effluent, cadmium was the most toxic and killed *Tetrahymena* at approximately a 10-fold lower concentration than needed to kill fish cells.

Development of fish brain cell lines and applications in toxicology. S.K. Walsh[1], M.A.R. Wright[1], M.P. Wilkie[1], R.C. Playle[1], N.C. Bols[2] and L.E.J. Lee[1]. [1]Department of Biology, Wilfrid Laurier University, Waterloo, ON; and [2]Department of Biology, University of Waterloo, Waterloo, ON.

Nervous tissue-derived cell cultures have proven invaluable tools in neurobiology and toxicology. They are used to test potential neurotoxicant compounds such as heavy metals, to study differentiation of neurons and glia, and to study aspects of neuronal regeneration and senescence. The teleost brain displays extensive adult neurogenesis and post-injury repair in brain tissues, necessitating further study of the cellular mechanisms behind these novel capabilities. Cell cultures from brain tissues of adult American eel (*Anguilla rostrata*), goldfish (*Carassius auratus)* and rainbow trout (*Oncorhynchus mykiss*) were initiated in the presence of Leibovitz's L-15 media supplemented with fetal bovine serum (FBS). A cell line (EelB) has been established that has retained the diploid chromosome number of the American eel (2n = 38). Cells appear large and epithelioid in nature with highly euchromatic nuclei and numerous lipid-based granules demonstrating yellow-orange autofluorescence. Immunocytochemical studies have shown reactivity to both anti-glial fibrillary acidic protein (an astrocyte marker) and anti-neurofilament (a neuronal marker), suggesting the cells' possible identity as neural cell progenitors. A goldfish cerebellar cell line and a trout brain cell line have also been initiated and are currently being characterized. The cell lines demonstrate differential responses to toxicants such as Cu, Al, Zn, to nutrients and growth factors, and could serve as models for neural regeneration studies as well as for elucidating mechanisms of neurotoxicity and neurophysiology.

Molecular and genomic endpoints/Marqueurs moléculaires et génomiques

Session chairs/Présidents: Brian Dixon and/et Lucy Lee

Fish and chips. J.B. Bruno, G.C. Van Aggelen, R. Skirrow and H.L. Osachoff. Environment Canada, Pacific Environmental Science Centre, North Vancouver, BC.

Toxicological testing is utilized to evaluate the potential for an effluent, or other material, to cause a measurable effect in a target organism. In the past, traditional endpoint measurements such as death, growth and reproduction were the chief means of determining the deleteriousness of an effluent or material. However, many effluents of historical concern, although no longer acutely toxic, still manifest toxicological effects on fish exposed to them in the wild. Since the last decade, genomics has revolutionized the entire field of biomedical research. While much publicity has been given to the health aspect of genomics research, it also has implications in environment toxicology. We are applying microarray analysis fundamentals to profile gene expression changes in aquatic animals exposed to contaminants. This combination of classical toxicology assays and expression profiling is termed toxicogenomics. Gathering large scale gene expression data is critical since we know that the maintenance of homeostasis is dependent on a multitude of genes and proteins. With this in mind, the Pacific Environmental Science Centre, with its collaborative partners, has developed a rainbow trout cDNA microarray incorporating genes involved in cellular physiology, including growth, immune response, endocrine regulation, metabolism and reproduction. This unique species-specific microarray is used to examine gene expression changes in organisms exposed to a variety of contaminants.

Application of molecular tools for linking metal toxicity and xenoestrogenic effects in coho salmon (*Oncorhynchus kisutch*). A. Tremblay[1], J.B. Bruno[2], H.L. Osachoff[2], R. Skirrow[2] and G.C. Van Aggelen[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Environment Canada, Pacific Environmental Science Centre, North Vancouver, BC.

Microarrays are fast becoming a preferred tool for the evaluation of organisms' changes in gene expression following contaminant exposure. They combine emerging technologies in genomics and bioinformatics to identify and characterize mechanisms of action of known and suspected toxicants. At Environment Canada's Pacific Environmental Science Centre (PESC), a rainbow trout cDNA microarray has been developed that can be used with additional Pacific salmonid species, such as chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*). The PESC rainbow trout microarray is composed of 207 cDNA gene transcripts representing broad gene classesprinted on aminosilane-coated Matrix ez-rays™ (glass slides). A standardized bioassay (96 h LC50) was conducted to investigate the effects of zinc, ethinylestradiol (EE2), and a zinc-EE2 combination on coho. Production of vitellogenin protein was assessed in whole fish blood using Best Checker Vitellogenin Kits (Cokey Co. Ltd., Japan) following the exposure to determine the estrogenicity of the treatment. PESC microarrays were then used to identify broad changes in gene expression. The gene expression profiles generated by this microarray technology revealed functional genomic responses to contaminant exposure. Quantitative PCR (using Stratagene Instruments MX3000P) will focus analysis of gene expression for a small subset of estrogen-responsive genes, providing validation of the results generated by microarray analysis. This three-pronged approach was designed to determine the link between metal toxicity and xenoestrogenic effects in a salmonid species. The genomic methods employed will be detailed using the results from this experiment.

Differential gene expression in Atlantic salmon (*Salmo salar*) exposed to saxitoxin using cDNA microarray and qPCR analysis. S.M. Bard[1], S. Zucchi[1], K. Goralski[2], M. Gubbins[3], J. Williams[4], R. Richards[4], B. Eddy[3], R.M. Stagg[5], S. Gallacher[5], C.J. Sinal[2], S. Douglas[4] and K.V. Ewart[4]. [1]Environmental Programmes, Dalhousie University, Halifax, NS; [2]Department of Pharmacology, Dalhousie University, Halifax, NS; [3]Department of Biological Sciences, University of Dundee, United Kingdom; [4]National Research Council of Canada, Institute of Marine Biosciences, Halifax , NS; and [5]Fisheries Research Service, Marine Laboratory, Aberdeen, United Kingdom.

The purpose of this study is to advance understanding of the effects of saxitoxin, a Paralytic Shellfish Toxin, on the differential gene expression in Atlantic salmon (*Salmo salar*). To examine saxitoxin detoxification pathways in the liver, we assessed expression of several important xenobiotic metabolizing and transport genes by QPCR: cytochrome P450 1A (CYP1A), glutathione S-transferase (GST), and P-glycoprotein (P-gp, a MDR1 homolog). 2 $\mu g \cdot kg^{-1}$ saxitoxin produced 3.4-fold induction of GST and 2.6-fold induction of P-gp as compared to the saline-treated control. Saxitoxin treatment did not effect gene expression neither of CYP1A nor the control gene β-actin. To investigate what other genes are affected by saxitoxin treatment, hepatic expression of 4000 different amplicons was assessed by microarray. Using this technology, we found 4 genes differently regulated. Two genes showed increased level of expression upon saxitoxin exposure: precerebellin-like protein (1.8-fold higher) and a gene of unknown function [sequence access number SL3-0194] (1.5-fold higher). While two other genes showed decreased level of expression: phosphoglycerate mutase (1.9-fold lower), and snake neurotoxin homolog (2.0-fold lower). These genes have not been previously associated with either detoxification or a general stress response. We are currently analyzing the hepatic expression of each of these genes in fish exposed to different saxitoxin doses and at different time points. Determination of the specific roles of these genes during saxitoxin exposure will lead to a better understanding of the cellular defense mechanisms that permit fish to tolerate the effects of natural product toxins produced during algal blooms.

Microarrays as a tool to study the effects of 17-α ethinylestradiol (EE2) exposure in the brain of goldfish (*Carassius auratus*). C. Martyniuk, H. Xiong, K.L. Crump, A. Nadler, E. Gerrie, A.J.

Woodhouse, X. Xia and V.L. Trudeau. Department of Biology, University of Ottawa, Ottawa, ON.

17-α ethinylestradiol (EE2), a potent estrogen used in birth control pills, is detectable in water systems worldwide and studies have documented effects in a number of aquatic species. Few studies to date, however, have investigated the effects of EE2 in the brain at a large-scale despite evidence that the brain of teleost fish may be more sensitive to estrogens than the mammalian brain due to a higher aromatase activity. Here we describe the production of a brain targeted goldfish (*Carassius auratus*) cDNA array and use this in conjunction with a mixed tissue carp microarray to study the genomic response to EE2. In March 2004, gonad-intact male goldfish were exposed to nominal concentrations of 29.64 $ng \cdot L^{-1}$ (0.1 nM) and 296.4 $ng \cdot L^{-1}$ (1.0 nM) EE2 for 15 d. Male goldfish treated with the higher dose of EE2 had significantly smaller gonads after the treatment period than control or the lower dose of EE2 ($p < 0.01$). Males had a significantly reduced level of circulating plasma E2 in both treatment groups ($p < 0.001$) as measured by RIA. Microarray and Q-PCR analysis identified brain aromatase mRNA as increasing at high concentrations of EE2 (>2 fold). Additional genes induced by EE2 were fibrinogen isoforms (alpha and gamma), proteins that act as mitogens in the brain. The use of microarrays in detecting estrogenic exposure is discussed. This information will be integrated into AURATUS: The goldfish environmental genome project (www.auratus.ca). (Supported by NSERC, CNTC and U Ottawa, OGS.)

Aquatic toxicity assessment using protein expression signatures from fish cell lines. L.E.J. Lee. Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Distinct protein expression signatures (PES) can be discerned from fish cell line proteomes at relatively simple, reliable, fast and inexpensive ways to monitor aquatic toxicity. Comparison of cellular sub-proteomes using cell lines could facilitate evaluation of toxicant action because of its sensitivity, replicability and ease of handling and manipulation. Proteome analysis has been touted to become a promising technique for evaluating organisms, tissues and cells by providing new information on biochemical changes. Unlike genomic profiling, the proteome is more representative of the function of specific cell types and proteomic profiling could be more useful for identifying changes in cells, tissues or organs. However, generating a database of the full cellular protein complement could be daunting and PES relies only on a subset of core protein profiles that could facilitate the analysis of intercellular differences as well as differential expression patterns as a result of physiological or environmental disturbances. This approach has been made with whole organisms and tissues of humans, mammals and lower vertebrates as well as invertebrates, but few reports have been made for discriminating the less complex and homogeneous cell line populations. Analysis of 2D gels from control and sample toxicants on fish cell lines will be presented that show distinct differences in protein spots which could be used as rapid signature profiling of toxicity.

Gene expression patterns in Arctic charr (*Salvelinus alpinus*) population contaminated by PCBs in northern Norway. M.M. Vijayan[1], S. Wiseman[1], E.H. Jørgensen[2] and A.G. Maule[3]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Norwegian College of Fishery Science, University of Tromso, Tormso, Norway; and [3]U.S. Geological Survey, Cook, WA.

Using a targeted trout cDNA microarray, we compared gene expression patterns in Arctic charr (*Salvelinus alpinus*) residing in two lakes, the Ellasjøen and Øyangen, on Bjornoya Island, Norway. These two lakes are only a few kilometers apart, but their contaminant loading is very different, thereby providing an ideal system to examine tissue gene expression pattern associated with long-term xenobiotic exposure. Studies showed Ellasjøen fish to have some of the highest ever recorded tissue levels of polychlorinated biphenyls (PCBs), whereas Øyangen fish had relatively low PCB levels. Liver tissues were collected from charr from both these lakes and total RNA extracted and hybridized

with our trout cDNA microarray. Indeed, cross-species comparison showed that this trout array cross-reacted nicely with salmonids, including Arctic charr. Several genes, including those related to stress, endocrine disruption and metabolism showed marked differences between samples from Ellasjøen and Øyangen. Cytochrome P450 1A (CYP1A) transcript levels were the highest in Ellasjøen fish and this was further confirmed using real-time quantitative PCR. Also, the CYP1A mRNA abundance was correlated positively with CYP1A protein content in the lake Ellasjøen fish. Overall, our results suggest that adaptation/adjustments to chronic contaminant exposure may involve the orchestration of stress-, metabolic- and endocrine-related genes in Arctic charr. The microarray technology has allowed us to identify and expand on pathways that were not normally associated with PCB contamination in fish. (This study was funded by the National Science Foundation, Polar Programs, the Norwegian Research Council and the Natural Sciences and Engineering Research Council of Canada Discovery grant.)

Newly identified p53 and p73 isoforms from *Mytilus*: potential biomarkers for oncogenesis. A.F. Muttray[1], R.L. Cox[2], C.L. Reinisch[2] and S.A. Baldwin[1]. [1]Department of Chemical and Biological Engineering, University of British Columbia, Vancouver, BC, and [2]Woods Hole Marine Biological Laboratory, Laboratory of Aquatic Biomedicine, Woods Hole, MA.

We are interested in the application of gene expression quantification as biomarkers of environmental effects. We are focussing on a well-characterized disease, haemic neoplasia, in two mussel species commonly used in environmental monitoring, *Mytilus edulis* and *M. trossulus*. It was demonstrated previously, using antibodies, that proteins of the p53 gene family are differentially expressed in healthy and leukemic mussels and clams. p53 is a classical tumor suppressor and has been studied extensively in mammalian systems. We have now isolated, sequenced and characterized several p53 gene family transcripts from *Mytilus*. One transcript is truncated at the 5' end of the sequence and resembles a DeltaNp73 identified previously in vertebrates. While the DeltaN isoform has been well characterized in mammalian species, this report is the first to identify this biologically important p73 isoform in any non-mammalian species and indicates a more ancient phylogenetic origin of this isoform than previously thought. In mammalian species, DeltaNp73 potently inhibits the tumor-suppressive function of p73 and p53, and its over-expression serves as a robust molecular marker for mammalian cancer. With the long-term goal of utilizing gene expression as a molecular marker for oncogenic transformation in marine molluscs, our studies are currently underway to determine leukemia-specific p53, p73 and DeltaNp73 expression. These genes potentially can be useful as biomarkers for environmental stress, especially if they are associated with a disease end-point.

Can gene expression in embryos of zebrafish (*Danio rerio*) predict chronic toxicity? K. Schirmer. Department of Cell Toxicology, UFZ – Center for Environmental Research, Leipzig, Germany.

The zebrafish (*Danio rerio*) is widely used in acute and chronic fish tests in ecotoxicology. As well, a toxicity test based on the zebrafish embryo (DarT – *Danio rerio* toxicity test) has recently been developed and implemented in Germany as an alternative to the acute fish test in effluent monitoring. Among the advantages of DarT are its simplicity and speed and the possibility to follow the development of the embryo due to its transparency. However, little is known about the expression of genes in zebrafish embryos and whether gene expression patterns could potentially be used to predict toxic effects in chronic fish tests. We thus exposed zebrafish embryos from 2 to 50 h post fertilization to the model substance 3,4-dichloroaniline (3,4-DCA), an industrial chemical that is also listed as priority pollutant. Based on a 14k-oligonucleotide microarray, 21 genes were identified to be significantly regulated by 3,4-DCA in embryos. The expression of four of these genes could be

confirmed by (quantitative) RT-PCR (cyp1a1, ahr2, hsp70, fzr1). Using (quantitative) RT-PCR, differential expression upon 3,4-DCA exposure was detected for three additional genes not present on the array but identified by literature search (nrf2, maft, hmox1). All of these genes were confirmed to be regulated to the same extent in early larvae (5 d post fertilization) but less of the 3,4-DCA was needed to detect this response. In fact, gene expression in early larvae was found to occur at 3,4-DCA concentrations similar to those required to detect toxicity in 30 day-old fish from an early life stage test. This give rise to the possibility to predict chronic toxicity based on gene expression early in zebrafish development. Keywords: microarray, gene expression, zebrafish, chronic toxicity, molecular endpoints

Sub-lethal doses of pesticides induce immunosuppression in Ontario anurans. B. Dixon[1], K.G. Droulliard[2], A. Albert[1], M-K. Gilbertson[2] and G.D. Haffner[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON.

The objective of this study was to determine whether the use of pesticides are suppressing the immune functions of the northern Leopard frog (*Rana pipiens*) and are therefore a contributory factor in the decline of frog populations. A suite of three non-lethal assays addressing the humoral cellular and innate branches of the immune system have been developed. The assays used are antibody response to keyhole limpet hemocyanin coupled to dinitrophenol (KLH-DNP), delayed type hypersensitivity and oxygen burst activity of neutrophils. The first stage was an injection study during which it was determined that sublethal doses (less than 1 $mg \cdot kg^{-1}$) of the pesticides dieldrin, malathion and DDT elicited severe immune alterations in adult frogs. The second phase was to collect frogs from various southern Ontario sites and then assess the immune function of each group. Significant differences in immune function were observed between sites two weeks after collection, but after 8 weeks in the laboratory most frogs' responses had reverted to the level of the control group. Contaminant analysis revealed that the frogs with the weaker immune responses had pesticide burdens similar to those used in the injections study. A feeding study showed that contaminants introduced through food courses were also capable of suppressing antibody responses at doses of less than 1 $mg \cdot kg^{-1}$. This effect could last for more than 34 weeks. Thus pesticides are altering immune function of southern Ontario frogs and may be contributing to declines.

Assessing thyroid-disrupting effects of estrogenic compounds on amphibian metamorphosis. N.S. Hogan, K.L. Crump, D.R.S. Lean and V.L. Trudeau. Department of Biology, University of Ottawa, Ottawa, ON.

The majority of research to date regarding endocrine disruption in wildlife has focused on the effects of estrogens on reproduction. However, the impact of estrogen on a different endocrine pathway, the thyroid system, is rarely considered. Such an interaction could significantly affect amphibian metamorphosis, a developmental process that is tightly controlled by thyroid hormones, receptors and enzymes. Studies in our laboratory with leopard frog tadpoles (*Rana pipiens*) indicate that chronic developmental exposure to xenoestrogens affects body weight and alters time-to-metamorphosis. To identify potential targets of estrogen on the thyroid axis, we developed a short term assay where tadpoles are exposed to ethinyl estradiol (EE2; 5nM; 48 h), followed by a challenge with triiodothyronine (T3; 5nM and 50nM; 48 h). Real-time RT-PCR was used to assess relative changes in transcript levels of TRá, TRâ, ERá and deiodinase enzymes (D2, D3) in whole brain and tail tissue. Pre-exposure to EE2 appears to modulate normal T3-stimulated regulation of deiodinase in the tadpole brain but has no effect on receptors in either brain or tail. These results indicate that deiodinase enzymes are targets of endocrine disruption by estrogens. Further examination of the

relationship between steroid hormones and thyroid status in amphibians is warranted.

Mechanisms of the action of the disruption in development and metamorphosis of *Rana pipiens* tadpoles by UV-B and 4-tert-octylphenol. M.C. Croteau[1], K.L .Crump[1], P. Duarte[1], M. Wade[2], D.R.S. Lean[1] and V.L. Trudeau[1]. [1]Department of Biology, University of Ottawa, Ottawa, ON; and [2]Health Canada, Environmental Health Sciences Bureau, Ottawa, ON.

Endocrine disrupting chemicals and UV-B radiation are factors that may contribute to amphibian declines. Though studies have shown that these stressors can affect the rate of development and metamorphosis of amphibians, the mechanism(s) of action behind this disruption remain unknown. Our study examines the effects of the estrogenic chemical 4-tert-octylphenol (OP) and UV-B on the thyroid system of *Rana pipiens* tadpoles, the ultimate mediator of amphibian development and metamorphosis. Gosner stage 25 tadpoles were exposed for 8 months to OP (2.06 $ng \cdot L^{-1}$, 2.06 $\mu g \cdot L^{-1}$), a 0.01% ethanol control, and a water control, with and without UV-B (about 22 $\mu W \cdot cm^{-2}$). Animals exposed to UV-B had delayed development (~1 month) compared to the other treatments. Only 2 to 4% of tadpoles exposed to UV-B metamorphosed compared to 6 to 12% in Controls and OP only groups. An interaction may exist between UV-B + 2.06 $\mu g \cdot L^{-1}$ OP because <1% of these tadpoles metamorphosed. Thyroid receptor (TR) α and β mRNA levels in tails were measured using multiplex real-time RT-PCR. TR β expression was upregulated ~1.7 fold in stage 29 tadpoles in both UV+OP groups compared to controls ($p < 0.05$). Expression of TR â in stage 34 tadpoles exposed to both concentrations of OP was upregulated about 2 to 3 fold compared to controls ($p < 0.05$). In another experiment, stage 25 tadpoles were exposed to UV-B (about 21 $\mu W \cdot cm^{-2}$) and histological analyses of the thyroid glands at stage 31 demonstrated no differences in gland morphology measurements between Control and UV-B groups. Further research is necessary to understand the molecular mechanisms behind the disruption in development and metamorphosis.

Aquatic ecotoxicity – metals and mining/Écotoxicité aquatique - métaux et exploitation minière

Session chair/Président: David Janz

A resident species approach to the development of a site-specific water quality criterion for cobalt in Panther Creek, Idaho. P.M. Mckee[1] and S.R. Hansen[2]. [1]Ecometrix, Brampton, ON; and [2]S.R. Hansen & Associates, USA.

A former copper-cobalt mine operated until 1982 in the Panther Creek watershed, within the Salmon River basin of Idaho. Panther Creek historically supported abundant populations of resident and anadromous salmonids, and various other fish species. By the 1950s and 1960s, acutely toxic conditions developed in the creek due to acid rock drainage and metal leaching from mine tailings and waste rock, contributing to the reduced fish and benthic invertebrate populations. These reductions in the biota were believed to be principally due to Cu; however, Co may have been a contributing factor. In 1994, the U.S. Environmental Protection Agency and an industry group agreed on a framework for remediation of the mine site and restoration of downstream salmonid populations, and major improvements in water quality and biological conditions have been achieved.

No state or federal criteria were available for aqueous Co concentrations, and available criteria in other jurisdictions are generally based on daphnid toxicity. Daphnid-based criteria were considered of questionable relevance to the protection of aquatic life in a high-gradient mountain stream. Accordingly, a procedure for establishing a site-specific clean-up criterion was developed using acute

and chronic toxicity tests carried out in Panther Creek water using rainbow trout (*Oncorhynchus mykiss*) and *Chironomus tentans*, combined with acute tests developed and carried out using three resident benthic invertebrates and a sculpin species. Completion of resident invertebrate tests required considerable trial and error to identify species amenable to capture, transport and maintenance in the laboratory. Candidate invertebrates were selected to include taxa sensitive to metals, which were abundant in the creek and capable of survival for a period sufficient to complete 96 h tests. Testing of the resident shorthead sculpin (*Cottus confusus*) was attempted but was not successful, leading to the use of a surrogate species, the mottled sculpin (*Cottus bairdi*). Acute-chronic ratios determined from trout and *Chironomus* tests were used to extrapolate chronic values for sculpin and resident invertebrates. Based on these various tests, a site-specific criterion of 0.1 $mg \cdot L^{-1}$ dissolved Co has been identified for the protection of salmonid early life stages and sensitive invertebrates.

Potential effect of complex metal mine effluent on the overwinter survival potential of juvenile fish. K.L. Driedger[1], L.P. Weber[2], C.J. Rickwood[1], C.I. Brereton[3], M.G. Dubé[4] and D.M. Janz[2]. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; [2]Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, SK; [3]INCO Ltd., Safety, Health and Environment, Copper Cliff, ON; and [4]Environment Canada, National Water Research Institute, Saskatoon, SK.

During summer, fish must allocate enough energy for growth as well as storage to prepare for winter. Environmental stress (e.g., temperature, disease and pollutants) increases the amount of energy individual fish need to allocate to basic metabolic processes and to repair damaged biological systems. The objective of this study was to evaluate the potential cumulative impact of two complex metal mining effluents and a municipal wastewater treatment plant in Junction Creek, Sudbury, Ontario on overwinter survival potential of fathead minnow (*Pimephales promelas*), creek chub (*Semotilus atromaculatus*) and white sucker (*Catostomus commersoni*) in their first year of life. Fish were collected from an upstream reference site (upstream of any direct effluent input points), downstream of the first mine effluent release, downstream of the second mine effluent release and downstream of a municipal wastewater treatment plant release. A second reference site was on a separate watershed and had no direct effluent release points. Measures of overwinter survival potential included weight-at-length, RNA/DNA ratio, muscle protein concentration and whole body triglyceride concentration. Creek chub exhibited decreased length and increased weight-at-length downstream from one mine site, and increased weight-at-length downstream from the second mine site. Increased length downstream from the first mine site and the municipal sewage treatment plant was observed in fathead minnows. Thus, growth of juvenile fish (not yet sexually mature) in Junction Creek was affected by treated mine and sewage effluent release points. Further work will focus on relating the alterations in growth with changes in biochemical measures of growth and bioenergetics.

Identifying the cause of aquatic toxicity to *Hyalella azteca* downstream of a uranium operation. E.L. Robertson and K. Liber. Toxicology Centre, University of Saskatchewan, Saskatoon, SK.

Past monitoring has noted benthic invertebrate community impairment downstream of the Key Lake uranium operation in northern Saskatchewan, Canada. The research presented here was aimed at identifying the cause(s) of this impact using a weight of evidence approach. Preliminary work in 2003 used a Sediment Quality Triad approach and confirmed the presence of an effect on benthic community structure, in addition to significant differences in surface-water, pore-water and whole-sediment chemistry. However, results from a whole-sediment toxicity test using *Hyalella azteca* indicated that sediment may not be the primary cause of benthic community impairment. Therefore, in the summer of 2004, *in-situ* and laboratory bioassays using *H. azteca* were conducted in addition

to the Sediment Quality Triad as part of a more broad investigation. Results from the *in-situ* and laboratory bioassays demonstrated that surface-water was the primary cause of acute toxicity to *H. azteca*. In the summer of 2005, new surface-water samples were collected for performing selected Toxicity Identification Evaluations. However, the 2005 samples were not acutely toxic to *H. azteca*. In consultation with industry, further investigation revealed that organic mill-process chemicals, which have been previously linked with sporadic effluent toxicity, were released during the in-situ experiment and sampling period of 2004. Results will be presented on the outcome of toxicity tests conducted to evaluate the hypothesis that organic mill-process chemicals were responsible for the toxicity observed in 2004.

Potential impacts of uranium milling effluent on juvenile fish bioenergetics, growth and overwinter survival. P .Bennett[1] and D.M. Janz[2]. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; and [2]Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, SK.

Survival of juvenile fish beyond their first year is critical for the maintenance of a healthy fish population. To evaluate possible uranium milling impacts on local aquatic systems, the bioenergetics, growth, and overwinter survival of juvenile northern pike (*Esox lucius*) and burbot (*Lota lota*) were examined at two uranium mines in northern Saskatchewan, Canada. At Key Lake uranium mine, burbot and northern pike from effluent exposed lakes were significantly higher in total body lipids and triglycerides compared to reference fish in both fall and spring; there was no difference in muscle RNA-DNA ratio or muscle protein. Exposed northern pike were not significantly different from reference fish in length, weight or condition factor; however burbot from the spring collection were higher in morphometric endpoints in exposed lakes. Interestingly, a decrease in lipids was not observed in either species following winter. Fall sampling at McClean Lake uranium mine revealed no significant differences in total body lipids or triglycerides, muscle RNA-DNA or protein between exposed and reference fish. Length, weight, condition factor and liver weight were not significantly different for northern pike in exposed versus control lakes; burbot were significantly heavier and longer in exposed lakes although there was no difference in either condition factor or liver weight. Summer work includes analysis of McClean spring 2005 samples, as well as analysis of morphometric and biochemical endpoints in slimy sculpin (*Cottus cognatus*). Results to date suggest possible alteration of energy allocation strategies for fishes at certain uranium mines in northern Saskatchewan.

Selenium toxicosis in northern pike (*Esox lucius*) exposed to uranium milling effluent. J.R. Muscatello[1] and D.M. Janz[2]. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; and [2]Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, SK.

Northern Saskatchewan is home to some of the top-producing uranium mines in the world. Elevated concentrations of Se have been reported in water, sediments and fish tissues downstream of certain uranium mining and milling operations, as a consequence of uranium ore extractions, dewatering and milling. Se is a contaminant of concern in receiving environments and may represent the most toxicologically relevant hazard to fish populations. The objective of the present study was to determine potential Se toxicosis in northern pike (*Esox lucius*) larvae exposed to uranium milling effluent at Key Lake uranium mine. Eggs and milt were stripped from adult female and male northern pike, fertilized in the field, and transported to the University of Saskatchewan. A two-way ANOVA design was employed with embryos originating from reference and exposure sites incubated in both reference and exposure water. Deformity analysis showed that fry originating from the exposure site (mean egg Se concentration 31.28 $mg \cdot kg^{-1}$ dry weight) exhibited a significantly higher incidence of deformities compared to those originating from the reference site (mean egg Se concentration 2.97

$mg \cdot kg^{-1}$ dry weight). Furthermore, there were significant positive linear relationships between egg Se concentrations and the frequencies of deformities observed in northern pike fry, as well as between egg and muscle Se concentrations in adult female pike. Our results suggest that maternal transfer of Se to eggs is the causative factor for the increased frequency of deformities found in northern pike fry at this uranium milling operation.

Assessment of various indicators of health in juvenile northern pike (*Esox lucius*) downstream of a uranium mill. J.M. Kelly[1] and D.M. Janz[2]. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; and [2]Department of Veterinary Biomedical Sciences, University of Saskatchewan, Saskatoon, SK.

Juvenile northern pike (*Esox lucius*) collected from lakes receiving effluent from the Key Lake uranium mill will be examined for potential effects on bioenergetic status due to effluent exposure. Levels of triglycerides in muscle and liver will be used to assess bioenergetics since these are the main forms of stored energy in fish. The triglyceride content of the main invertebrate and vertebrate prey items (spottail shiners (*Notropis hudsonius*), chironimids, and larval dragonflies) will be evaluated to establish a possible link between pike bioenergetic status and diet quality. Past research demonstrated that pike exposed to uranium milling effluent had higher lipid stores than reference fish; an examination of prey items may help elucidate a possible explanation for this unanticipated result. The effluent is largely comprised of different metals and metalloids, some of which have been related to increased production of reactive oxygen species in biological systems. As an indirect measure of oxidative stress, the activity of glutathione peroxidase and superoxide dismutase, two major antioxidant enzymes, will be quantified in liver and kidney tissue. The level of glutathione, an important scavenger of reactive oxygen species, will be measured as well.

Sources of elevated mercury concentrations in fish from the St. Lawrence River at Cornwall? A.R. Fowlie[1], P.V Hodson[1] and B.M.C. Hickey[2]. [1]Department of Biology, Queen's University, Kingston, ON; and [2]St. Lawrence River Institute of Environmental Sciences, Cornwall, ON.

The Cornwall river waterfront (St. Lawrence River, Ontario, Canada) has received inputs of mercury from decades of industrial pollution. Identified as an "Area of Concern" by the International Joint Commission, the sediments from depositional zones along the waterfront are contaminated with mercury. Methyl mercury (MeHg) concentrations in fish downstream from these sources are also elevated, but it is not clear whether sediment contamination is the primary source. We are investigating MeHg transfer through aquatic food chains at reference and contaminated sites. Preliminary evidence indicates that MeHg bioaccumulated in yellow perch (*Perca flavescens*) as small as 11.2 cm to levels exceeding Canadian human consumption guidelines ($0.2\ mg \cdot kg^{-1}$). Surprisingly, fish from a contaminated upstream zone contained more MeHg than fish from a contaminated zone only 2 km downstream. This trend was also confirmed with other fish species such as rock bass (*Ambloplites rupestris*) and brown bullhead (*Ictalurus nebulosus*). Possible reasons could include differences among sites in food web complexity (more trophic transfers and biomagnification), differences in the fish growth rate and population structure, or a local source at the upstream site, including conditions that would favour mercury methylation (such as pockets of organic enrichment). To test these hypotheses we are using multiple lines of evidence that include a description of the aquatic food web as indicated by fish stomach contents, and benthos and fish sampled from each site. Trophic level will be assigned by stable nitrogen and carbon isotope analyses, and components of the food web (fish and invertebrates) will be analyzed for MeHg.

Aquatic ecotoxity – chemistry/Écotoxicité aquatique - chimie

Session chair/Président: Dieb Birkholz

A chronic toxicity identification and evaluation (TIE) method for pulp and paper effluent. P.F. Lee, J. Joncas, D. Kwiatkowski, K. Maa and S. Walford. Department of Biology, Lakehead University, Thunder Bay, ON.

Although pulp and paper mills in Canada routinely pass acute toxicity tests, chronic tests often suggest significant effects from mill effluent. As mills become involved in identification of cause studies, there is a need to characterize the chronic toxicants. We are currently in the pilot phase of the development of chronic toxicity identification and evaluation (TIE) procedures for pulp and paper effluent. Using *Ceriodaphnia dubia* as an indicator organism, we have modified standard TIE protocols developed by the EPA. Phase 1 of our TIE procedure, broadly characterizes the cause of toxicity by modifying the effluent with various treatments (example – organic removal). Phase 2 of a TIE further narrows down the characteristics of the potential toxicants until the specific cause is isolated. The problem with pulp and paper effluent is that it is a very complex mixture of both natural components from the wood and added substances from the pulping process. In order to conduct the TIE we use preliminary separation and extraction of the major groups of compounds. We have found the best way of doing this is to utilize HPLC to progressively fractionate the effluent followed by testing with *Ceriodaphnia*. Final identification of compounds uses both GC-MS and NMR instrumentation. Our initial results from a variety of mill samples suggests that various resin fatty acids are the main cause of any observed chronic toxicity.

New methods in QSAR: use of structural equation modeling to describe PAH photoinduced toxicity. M. Lampi, M. Reynolds, D.G. Dixon and B.M. Greenberg. Department of Biology, University of Waterloo, Waterloo, ON.

Recent advances in the technological fields related to computing have led to a large increase in the use of sophisticated modeling techniques. Ecotoxicology is no different, and indeed there has been wide application of such technology. A recent introduction to the field has been the use of complex methods, particularly neural network models. A complementary technique that is not widely employed in ecotoxicology is structural equation modeling (SEM). This method encompasses many statistical techniques including regression, factor and path analysis. Structural equation modeling allows for testing of hypotheses regarding relationships between observed and latent (unobserved) variables. Latent variables are theoretical concepts that unite phenomena under a single term, and are expressed in terms of directly measured variables. A novel application of structural equation modeling was used to validate the assumption of a bipartite mechanism for photoinduced toxicity of PAHs that includes photosensitization and photomodification. Two latent variables were created to represent the processes of photosensitization and photomodification, which both contribute to phototoxicity. These were based solely on physicochemical and photodynamic properties of the PAHs. The use of SEM enables the weighting of these properties, and their contribution to each latent variable individually, as well as the contribution of the latent variables to toxicity. Six existing PAH phototoxicity data sets were used and structural equation models were estimated using SEM software. These models accounted for a high amount of variance in six different sets of PAH phototoxicity data from different organisms, while providing insight regarding the contribution of different physicochemical and photodynamic descriptors to toxicity. The flexibility of SEM is evident as the relative contributions of each factor could be determined and compared. This study illustrates the promise for this type of modeling in ecotoxicology, of which potential future uses include assessment of synergism, and to predictive models developed for other contaminants.

Identification of toxic halogenated tolyltriazoles in industrial effluents. S.E. Goudey[1], L.N. Taylor[2] and D.A.Birkholz[3]. [1]HydroQual Laboratories, Calgary, AB; [2]Stantec Consulting, Guelph, ON; [3]Enviro-test Laboratories, Edmonton, AB.

Rainbow trout (*Oncorhynchus mykiss*) bioassays were performed on industrial effluent obtained from a polyethylene plant, two straddle gas plants, a fertilizer plant and a tire manufacturer. All effluents were found to be toxic to rainbow trout and subsequent toxicity identification evaluations determined the source of toxicity to be cooling water blowdown. Compounds identified as the cause of toxicity were determined to be bromo-, dibromo-, tribromo-, chloro-, dichloro-, bromochloro-, bromodichloro-, and dibromochlorotolyltriazoles. Toxicity testing using luminescent bacteria, Fathead minnow (*Pimephales promelas*) and rainbow trout revealed that bromotolyltriazoles were the most toxic and that chlorotolytriazoles were the least toxic. A cooling tower simulation was performed and brominated tolyltriazoles were isolated and fractionated using HPLC. Subsequent toxicity testing revealed that dibromotolyltriazoles were most toxic, however both bromo- and tribromo-tolyltriazoles were also toxic. Total concentration of brominated tolytrizoles was estimated to be 110 $\mu g \cdot L^{-1}$ and gave an observed toxicity of 5.0 TU using rainbow trout. The source of halogenated tolyltriazoles depends upon what type of corrosion inhibitors and biocides are used in cooling tower management. These compounds are persistent and may be endocrine disrupting.

Aquatic ecotoxicity – general/Écotoxicité aquatique - générale

Session chair/Président: Mohan Kohli

Toxicity of ethylbenzene to *Daphnia magna* in a flow-through system. C.J. Kennedy[1] and N.K. Nagpal[2]. [1]Department of Biological Sciences, Simon Fraiser University, Burnaby, BC; and [2]British Columbia Ministry of Water, Land and Air Protection, Victoria, BC.

The toxicity of volatile organic compounds to aquatic organisms by standard test methods is complicated by loss of the chemical from test water. A chemical delivery system was designed specifically to address this problem using ethylbenzene (EB) as a model compound. A Transduction LSI 11/02 compatible computer using a custom built interface and proprietary software allowed for the generation of different EB concentrations in enclosed glass vessels. Using computer-controlled pumps and solenoid valves, an appropriate amount of EB stock solution and conditioned water were delivered to each vessel every 4 h, replacing 54 L of water daily in each vessel while maintaining the desired toxicant concentration. Individuals of the freshwater invertebrate *Daphnia magna* were housed within specially designed glass tubes equipped with nytex "windows" which allowed viewing of animals at appropriate observation times as well as a consistent flow of contaminated water through the tube. Both the acute and chronic toxicity of EB to *D. magna* was determined using this system. The 48 h EC50 value (2.20 $mg \cdot L^{-1}$) was 18% lower than previously reported literature values. In chronic trials, neonates were placed in vessels and fed daily with a standard algae and yeast suspension. Survival and brood production was observed daily through 28 days. Reproduction was significantly inhibited at 0.5 $mg \cdot L^{-1}$ compared to controls. Reproductive impairment included fewer broods and lower numbers of neonates per brood. These data may be used in British Columbia water quality guideline derivation.

Effects of two pesticides, chlorothalonil and chlorpyrifos, on imidacloprid toxicity to *Chironomus tentans*. S.J. Stoughton[1], K. Liber[1], J.M. Culp[2] and A. Cessna[3]. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; [2]Environment Canada, National Water Research Institute, Fredericton, NB; and [3]Environment Canada, National Water Research Institute, Saskatoon, SK.

Imidacloprid is a relatively new nicotine mimic insecticide that is widely used across North America. Imidacloprid residues are routinely found in surface waters near treated fields following periods of spray drift, surface runoff, or subsurface water flow. Pesticides such as chlorpyrifos, an organophosphate insecticide with a complementary mechanism of action, and chlorothalonil, a fungicide with a completely different mechanism of action, are often used concurrently with imidacloprid, and consequently residues may be found together in aquatic systems. Pesticides in mixtures can interact in many ways depending on their mechanisms of action, yet there are no toxicity data available for pesticide mixtures containing imidacloprid. Previous tests have shown that imidacloprid significantly inhibits 10 d survival and growth of *Chironomus tentans* larvae at a concentration of about 2.4 $\mu g \cdot L^{-1}$. Using this information, 4 d and 10 d toxicity tests were run using binary mixtures of imidacloprid (1 or 3 $\mu g \cdot L^{-1}$) and either chlorothalonil or chlorpyrifos at 0.3X or 1X of their respective no observable effect concentration values. These binary mixture toxicity tests were used to determine if low concentrations of chlorothalonil and chlorpyrifos enhance or have no effect on both acute and chronic imidacloprid toxicity to *C. tentans* larvae.

Assessment of mixture toxicity of metals, PAHs and oxy-PAHs in *Daphnia magna*. F. Xie[1], M. Lampi[1], D.G. Dixon[1], W.P. Norwood[2], U. Borgmann[2], X. Huang[1] and B.M. Greenburg[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON; and [2]Environment Canada, National Water Research Institute, Burlington, ON.

Metals, polycyclic aromatic hydrocarbons (PAHs) and their derivatives are commonly present in complex mixtures in the environment. Strikingly, little is known about mixture toxicity and specific mechanisms of these contaminants. Cu, Ni and Cd were selected, representing a highly redox-active, a moderately redox-active and a non-redox metal, respectively. We have found synergistic effects for the combinations of Cu or Ni with phenanthrenequinone (PHQ), a major photoproduct of phenanthrene (PHE). In contrast, additive toxicity was observed in mixtures of Cd plus PHQ, as well as in mixtures of Cu plus PHE. The increased toxicity of Cu plus PHQ is physiologically based because assimilation of Cu was similar with or without PHQ. We have show that Cu and Ni alone induced reactive oxygen species (ROS) formation in *Daphnia magna*, whereas PHQ and Cd had no effect on ROS production using the dicholorofluorescein (DCF) fluorescence assay. Relative to the individual metals, enhanced ROS production was observed in *D. magna* treated with mixtures of Cu plus PHQ, and Ni plus PHQ combination, but not with Cd plus PHQ mixtures. Furthermore, the increased toxicity and ROS production were attenuated by antioxidants L-ascorbic acid, β-carotene or dimethylthiourea. The results indicate that there are potential toxic interactions between redox active metals and modified PAHs, and provide evidence for a ROS mediated mechanism for mixture toxicity.

***Clostridium botulinum* Type E beurotoxin in Great Lakes fish: implications for transmission of avian botulism.** R.D. Moccia[1], A. Yule[1], I.K. Barker[2] and J. Austin[3]. [1]Aquaculture Centre, University of Guelph, Guelph, ON; [2]Department of Apthobiology, University of Guelph, Guelph, ON; and [3]Health Canada, Bureau of Microbial Hazards, Ottawa, ON.

Since 1999, large-scale mortalities of fish-eating birds (common loons (*Gavia immer*); red-breasted mergansers (*Mergusser rator*); grebes (*Podiceps spp.*)) attributable to Type E botulism have been observed on the lower Great Lakes, especially Lake Erie. The mechanism of Type E botulism exposure in fish-eating birds is unknown. We report here, the use of a fish botulism exposure model to compare the sensitivity of rainbow trout (*Oncorhynchus mykiss*), round goby (*Neogobius melanostomas*), walleye (*Sanders vitreus*) and yellow perch (*Perca flavescens*) to *Clostridium botulinum* neurotoxin Type E (BoNT/E). Each species expressed a unique combination of clinical

signs, consisting of changes in behaviour and/or skin pigmentation, prior to death. Behavioural changes varied among species, but included increased respiration rate; opercular abduction; intermittent bursts of agitated swimming followed by periods of immobility; loss of coordinated fin control; transient periods of inversion; head-up orientation; terminal loss of voluntary motor function; and complete paralysis. Free toxin was sought by mouse bioassay in muscle tissue and the remainder of the carcass of each fish collected at the end of the experiment. Free toxin was present in muscle tissue of only 2 of 48 fish. However, many non-muscle carcass samples contained detectable free toxin. Results of this study support the contention that live, intoxicated fish can represent a vector for transfer of BoNT/E to birds, since intoxicated fish containing free BoNT/E, can survive for up to several days, depending on species and toxin dose. Furthermore, intoxicated fish might be preferentially selected as prey by fish-eating birds, due to changes in behaviour and/or appearance that make them more conspicuous, and probably easier prey, in comparison with non-intoxicated conspecifics.

Making sense of fish estrogenicity data from refinery experiments. 1. Design and data generation. J.P. Sherry[1], B.A. Zajdlik$_2$, J.L. Parrott[2], S. Munro[3], T. Kierstead[4] and T.S. Moran[4]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]B. Zajdlik and Associates, Rockwood, ON; [3]Sarnia-Lambton Environmental Association, Sarnia, ON; and [4]Pollutech Enviroquatics Ltd., Point Edward, ON.

We used a two-prong strategy to investigate the potential of wastewaters from several Ontario refineries to induce estrogenic responses in juvenile rainbow trout (*Oncorhynchus mykiss*). The potencies of end-of-pipe wastewaters were tested in 21 d bioassays using a static full-renewal design. Caged trout were used to assess the recipient waters for estrogenicity and anti-estrogenicity in a pilot experiment. Estrogenicity was measured as induced vitellogenin (Vg) in the plasma of the exposed fish. Anti-estrogenicity was measured as depressed induction of Vg in 17δ-estradiol primed RT. EROD was measured in the livers of the treated trout as a measure of exposure to polynuclear aromatic hydrocarbons (PAHs) and related compounds. The condition factor (CF) and liver somatic (LS) indices of the treated fish were also calculated. A preliminary analysis of the data showed that wastewater from one of the refineries caused a repeatable estrogenic response in the laboratory bioassay. The caged fish showed no evidence of biologically meaningful responses; although there was marginal induction of Vg in the case of one refinery.

Log bundling strand recycling initiative – the road to success. J.E. Wilkinson. Environment Canada, Environmental Protection Service, Vancouver, BC.

In 2001, Environment Canada began working with the forest industry to develop the Log Bundling Strand Recycling Initiative. Each year, over 15 million metres (50 million feet) of log bundling strand is used by coastal logging operations in British Columbia. This public-private sector initiative has helped to ensure that log bundling strand is handled in an economically viable and environmentally responsible manner. Since the inception of this initiative, the forest industry's handling of bundle strand has evolved dramatically. In 2001, Environment Canada performed an industry wide audit of strand use and found that less that 24% of strand used by British Columbia's coastal forest operations was being recycled. In December 2004, Environment Canada released a report demonstrating that over 80% of strand used is being recovered and many industry leaders have incorporated Best Management Practices into their business plans. This dramatic improvement benefits the environment, communities, and the bottom line. Now that Environment Canada has completed the third and final phase of this initiative, it is time to share the success story and detail the great accomplishments that can be achieved through public-private sector initiatives.

Lake Opeongo, Algonquin Park: environmental trends in hydroxylated PCBs. L.M. Campbell[1], U. Daisuke[2], C. Darling[2], D.M. Whittle[3], M. Arts[2] and M. Alaee[2]. [1]Department of Biology, Queen's University, Kingston, ON; [2]Environment Canada, National Water Research Institute, Burlington, ON; and [3]Department of Fisheries and Oceans, Bayfield Institute, Burlington, ON.

Hydroxylated PCBs (OH-PCBs) are an emerging class of contaminants in the Great Lakes region, and OH-PCBs are known to be thryoidogenic and may be contributing to thyroid microfollicular hyperplasia in Great Lakes salmonids. OH-PCBs also may affect human and mammalian biochemical processes dependent on thyroid hormones. Sources of OH-PCBs to lakes are still unknown, and it has been argued that a combination of internal metabolic processes, previously-metabolized sources (i.e., sewage) and abiotic transformations may contribute to OH-PCB burdens in fish from the Laurentian Great Lakes. Lake Opeongo, a large lake in Algonquin Provincial Park, is an important canoeing and fishing destination. The major source of contaminants to this lake is primarily atmospheric, and sewage and other important previously-metabolized sources of contaminants are not prevalent in this lake, unlike for Lake Ontario. In order to determine importance of atmospheric sources, food chain biomagnification and internal metabolic processes to OH-PCB burdens in fish, water, snow as well as the pelagic food chain, zooplankton, cisco (*Coregonus artedii*), lake trout (*Salvelinus namaycush*) and burbot (*Lota lota*), were sampled in the fall of 2003. Stable isotopes and fatty acids were measured to determine food web linkages and biomagnification. OH-PCB congener distributions and homolog groups were measured and compared in the various matrices.

Sensititivy of mummichog (*Fundulus heteroclitus*) to endocrine disruptors during different reproductive periods. K. Shaughnessy and D.L. MacLatchy. Department of Biology, University of New Brunswick, St. John, NB.

Recent laboratory exposures using mummichog (*Fundulus heteroclitus*) to putative compounds or mixtures functioning as endocrine disruptors have indicated that fish may have seasonal sensitivities. To investigate this hypothesis, an analysis of testosterone levels in fish exposed to various putative endocrine disruptors (including complex effluents) carried out in our laboratory between 1997 and 2004 was conducted. The primary objectives of the analysis were to provide information on the basic reproductive biology of mummichog held in the laboratory, and to determine if differences in reproductive state and/or time of exposure influence mummichog sensitivity to contaminants. The analysis revealed that mummichog are reproductively active from mid-March until mid-August in the lab. Plasma testosterone levels did not correlate with gonad size. Lunar cues appear to influence circulating testosterone levels in male mummichog, such that peak levels correlate with the full moon. Moon phase did not correlate with gonad size for either sex during the spawning season. Mummichog with large gonads responded to contaminants less often than fish with smaller gonad sizes, indicating that mummichog sensitivity to exposure can change depending on the reproductive state of the fish. From this data analysis, recommendations for experimental designs can be suggested.

A preliminary assessment of the potential effects of seafood processing effluents on the aquatic environment. [1]B.L. Jamieson[1], L. Park[2], G. Gagnon[1] and M. Walsh[1]. [1]Department of Civil Engineering, Dalhousie University, Halifax, NS; and [2]Department of Fisheries and Oceans, Oceans Division, St. John's, NL.

The North American seafood processing industry is currently facing significant challenges to maintain productivity and competitiveness in an evolving global market while improving wastewater handling management strategies due to more stringent environmental legislation. Wastewater generated from seafood processing operations are typically characterized as having elevated concentrations of organic matter, total suspended solids, and nitrogen as ammonia. Consequently, the

direct discharge of these waste streams has the potential to cause localized depletions of dissolved oxygen, aesthetically degrade receiving waters through eutrophication and be acutely toxic to fish. However, critical examinations into the potential effects of these wastewater streams on the aquatic environment have been limited due to the wide variability in effluent contaminant concentrations associated with plant process design, water consumption demands and production schedules. The purpose of the present investigation was to conduct a preliminary assessment of the potential effects of seafood processing effluents on the aquatic environment. In particular, effluent samples from three shell- and finfish processing operations in Atlantic Canada were collected and characterized for biochemical oxygen demand (BOD_5), chemical oxygen demand, turbidity, total suspended solids, ammonia (NH_3/N_4^+-N) and total organic halides. Bench-scale testing of coagulation with alum and dissolved air flotation were conducted with each effluent sample to examine contaminant reduction capabilities of these wastewater treatment technologies and resultant impacts to effluent toxicity. Aquatic toxicity of the untreated and treated effluents were evaluated using the acute lethality test employing threespine stickleback (*Gasterosteus aculeatus*) and Microtox® bioassays. It is anticipated that the results of this study will provide novel scientific findings and a constructive framework for the seafood processing industry, regulatory agencies and the research community to assess the effects of these waste streams on receiving water bodies and evaluate potential mitigation strategies.

Progress on the ecological categorization of "unknown or variable composition, complex reaction products" (UVCBs) on the Domestic Substances List. A. Okonski, M. Lin, S. Schnabel, D. MacDonald, P. Robinson, D. Morin and P. Costa. Environment Canada, Existing Substances Branch, Gatineau, QC.

The Domestic Substances List (DSL) consists of about 23,000 substances, 4400 of which are "unknown or variable composition, complex reaction products, and biological materials" (UVCBs). These substances must be categorized by September 2006 (CEPA 1999). Environment Canada developed a strategic approach for UVCB ecological categorization. First, UVCBs were sorted into six major streams: organics, organometallics, organic metal salts, inorganics, polymers, biologicals. Since approaches for the ecological categorization of non-UVCB substances (e.g., discrete organics, organometallics, inorganics) have already been developed, they are implemented to categorize UVCBs, falling into these groups. Further, UVCBs were separated into two categories: (i) low ecotoxicological concern (850 substances); and (ii) under review for persistence, bioaccumulation, and aquatic toxicity (PBiT) – UVCBs, requiring further considerations (3550 substances). Experimental information was used to identify P, B, iT pivotal values (175, 33, and 296 UVCBs, respectively). For about 800 UVCBs, representative molecular structures were derived, and modeled PBiT data were produced. Category approaches were developed to identify sub-groups of UVCBs with similar structures and properties, where the ecotoxicity and environmental fate of the substances within such sub-groups would be assumed to be similar. Therefore, when data are available for some substances from the sub-group, they are used to categorize the whole sub-group. To date, Environment Canada made categorization decisions for 1400 UVCBs, for which profiles were required, and work is on-going on others.

Toxicity investigations on a pit-lake in northwestern Ontario, Canada. A.R. Goold and P.F. Lee. Department of Biology, Lakehead University, Thunder Bay, ON.

Investigations are continuing on the cause of toxicity in Hogarth Pit-Lake near Atikokan, Ontario, Canada. This lake is nearly devoid of life compared to the nearby Caland Pit-Lake which has an active aquaculture operation in the upper levels of this meromictic lake. Detailed chemical analyses showed that Hogarth Pit Lake was high in sulfates but did not have elevated levels of heavy metals

in its near neutral water column. However, seeps entering Hogarth have varied water quality. Standard LC50 tests have revealed that the main water body of the pit-lake is not acutely toxic but the paucity of life suggested a chronic problem. Detailed TIE analyses using a chronic indicator for toxicity are continuing to isolate the most likely cause(s) of restricting the development of a normal food chain in Hogarth. Results from these analyses will be applied to an ecological risk assessment in the area. The research is critical since continuous rising waters in these pit-lakes will cause outflow into the adjacent Seine River system, predicted to occur in 2030.

Environmental risk assessment as a tool for managing contaminated sediments in Victoria Harbour: refining risk estimates with field studies. S. Weech[1], D.A. Bright[1], J. Hansen[1], R. Reid[2] and R. MacDonald[2]. [1]UMA Engineering Ltd., Victoria, BC; and [2]Transport Canada, Vancouver, BC.

In support of harbour management and potential divestiture, an environmental risk assessment was completed to provide information on the types of contaminants in the study area, the spatial and vertical distribution of contaminants in sediment, uptake into biota, and estimated risks to ecological receptors. Based on a screening against ambient/background contaminant concentrations, the primary contaminants associated with sediments in the harbour include metals (Cu, Pb, Hg, Zn, Sb and Ag), PAHs and PCBs. Concentrations of contaminants of potential concern (COPCs) were measured in dietary biota (plankton, shrimp, crabs, fish, mussels and clams) and subsequently used in risk modelling for potential wildlife receptors. Three mammalian and seven bird species were identified as potential receptors of concern to cover a wide range of dietary habits and exposure scenarios. The major findings in terms of ecological risk were: (i) mammals and piscivorous birds do not appear to be at risk of dietary COPC exposure; (ii) PAHs do not pose a risk to wildlife receptors under any exposure scenario; (iii) birds consuming a more varied diet (i.e., crustaceans, fish and bivalves) are at increased risk of methylmercury and PCB exposure; and (iv) non-migratory birds feeding on intertidal bivalves (e.g., black oystercatcher) are at greatest risk. These results, combined with results of the human health risk assessment and benthic community analysis, will be used to develop future risk management strategies.

Evaluation of the environmental safety of a proposed ballast water biocide: Peraclean® Ocean. J.R. Elphick[1], K.L. Bergmann[2], M. Liebl[2], C.M. Pidgeon[2], B. Hopf[3] and B. Gec[4]. [1]Nautilus Environmental, Victoria, BC; [2]Nautilus Environmental, Tacoma, WA; [3]Degussa AG, Hanua-Wolfgang, Germany; and [4]Degussa AG, Burlington, ON.

Introduction of non-indigenous species is a risk associated with discharge of ballast water from ships transporting cargo between regions. Consequently, technologies to treat ballast water to destroy or remove organisms are being evaluated. Degussa AG has developed a product, PERACLEAN® Ocean, which appears to have suitable characteristics as a ballast water biocide. The purpose of this study was to evaluate the environmental safety of this product according to guidelines prepared by the Washington Department of Ecology. Toxicity tests were conducted with four species. Larval development of the mussel, *Mytilus galloprovincialis*, was the most sensitive test, with an EC50 of 3.6 $mg \cdot L^{-1}$ product. Toxicity degraded rapidly, with 86% dissipation of toxicity within 48 h under conditions of 8 ± 1 °C and darkness. Dissipation of toxicity corresponded to degradation of peracetic acid; the relatively small amount of toxicity present after 48 h was caused by residual hydrogen peroxide. The characteristics of high initial toxicity and rapid dissipation of toxicity are beneficial attributes for a ballast water biocide and suggest that PERACLEAN® Ocean may provide a suitable treatment system from an environmental safety standpoint.

Evaluation of inter- and intra-laboratory variability of toxicity test methods using Pacific

herring (*Clupea harengus pallasi*). J.R. Elphick[1], P.A. Dinnel[2], K.L Bermann[3], H.C. Bailey[4], L.N. Paisano[2] and R. Marshall[5]. [1]Nautilus Environmental, Victoria, BC; [2]Shannon Point Marine Center, Anacortes, WA; [3]Nautilus Environmental, Tacoma, WA; [4]Nautilus Environmental, San Diego, CA; and [5]Washington State Department of Ecology, Olympia, WA.

Efforts to evaluate and protect Pacific herring (*Clupea harengus pallasi*) stocks in Washington State have resulted in a demand for aquatic toxicity tests using this species for application in National Pollutant Discharge Elimination System (NPDES) permits to ensure that discharges from industries and other point sources are not having adverse effects on this fish species. Consequently, test methods using early life-stages of Pacific herring have been developed, including a 96 h survival test, an 18 d embryonic development test, and a 10 d larval growth and survival test. This presentation summarizes the results of tests conducted in a commercial laboratory for each of the three test methods using three reference toxicants, and compares them with results from tests conducted previously in the laboratory where the methods were developed. Thus, these data provide an indication in inter- and intra-laboratory variability associated with the test methods. In addition, the sensitivity of the herring tests to the three reference toxicants, as well as two other toxicant mixtures, are compared with results from a suite of other standardized toxicity tests that have been applied in NPDES permits.

Effects of pulsed water-borne exposure to atrazine, hexazinone and nonylphenol mixtures on growth and survival of Atlantic salmon smolts (*Salmo salar*). W.L. Fairchild[1], J.T. Arsenault[1], D.L. MacLatchy[2], K. Haya[3], L.E. Burridge[3], and S.B. Brown[4]. [1]Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB; [2]Department of Biology, University of New Brunswick, St. John, NB; [3]Department of Fisheries and Oceans, St. Andrews Biological Station, St. Andrews, NB; and [4]Environment Canada, National Water Research Institute, Burlington, ON.

Prior research has shown that short-term, water-born exposures of Atlantic salmon (*Salmo salar*) smolts to environmental levels of 4-nonylphenol affect parr-smolt transformation (PST) such that subsequent growth in seawater is impaired and plasma Insulin-like Growth Factor-1 (IGF-1) concentrations are reduced. Recent results in the United Kingdom have indicated significant mortality on transfer to sea water in smolts exposed to atrazine, and nonylphenol/atrazine mixtures continuously for 7 d. Our study was conducted to determine the effects of low-level, episodic water-borne exposures of 4-nonylphenol, atrazine, hexazinone, and mixtures of 4-nonylphenol and atrazine or hexazinone, on smolt growth and survival. Smolts were exposed to environmentally-relevant (20 $\mu g \cdot L^{-1}$, each constituent) 24 h pulses separated by 5 d. Treatments occurred in mid-May in fresh water, during the final stages of PST. Smolts were then gradually transferred to sea water and subsequent smolt growth and survival was evaluated. The 4-nonylphenol, nonylphenol/atrazine and atrazine treatment groups had a higher proportion of small smolts in July than did the control group. Atlantic Canadian estuaries and rivers have been shown to have sources, and measurable concentrations of both 4-nonylphenol and pesticides. Smolt growth in the first months at sea and smolt plasma IGF-1 concentration, have been positively related to the rate of subsequent salmonid adult returns.

Effects of pesticides and nonylphenol on growth, histology, gene expression and survival of lobster larvae. W.L. Fairchild[1], J.T. Arsenault[1], M. Comeau[1], K. Benhalima[1], K. Haya[2], L.E. Burridge[2], and P.M. Jackman[3] and K.G. Doe[3]. [1]Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB; [2]Department of Fisheries and Oceans, St. Andrews Biological Station, St. Andrews, NB; and [3]Environment Canada, Environmental Conservation Branch, Moncton, NB.

Some currently used pesticides target insect hormone function to interfere with molting and chitin

physiology. Crustaceans, including the American lobster (*Homarus americanus*) use similar hormone systems to control their molt. If pesticides, or other chemicals, that can act on molting and chitin physiology are present in the environment in sufficient quantities, during critical developmental periods, the potential for effects on lobster exists. In this study, the effects of low-level, water-borne exposures of 4-nonylphenol and pesticides on the molting success and survival on lobster larval Stages II to IV were studied. In addition, surviving lobster were sampled and preserved for histology, and for genomics DNA microarray analyses, at various times throughout the tests. Lobster larvae reared at Marine Centre, Shippagan, NB, were transported to Environment Canada, Moncton, NB, for testing. Long term toxicity tests of 6 week duration with multiple exposure concentrations were conducted in 2004 and 2005. Larvae were reared individually, fed daily, and had exposure solutions renewed 3 times a week. Long term tests were conducted with 4-nonylphenol and tebufenozide in 2004 and with 4-nonylphenol and atrazine in 2005. Short term acute tests were also conducted on tebufenozide, diflubenzuron, endosulphan and hexazinone in 2005. Regional estuaries and rivers have been shown to have sources, and measurable concentrations of both pesticides and 4-nonylphenol.

Toxicity examination of phenanthrenequinone to model organism *Vibrio fischeri*, a reactive oxygen species mediated mechanism? W. Wang, D.G. Dixon and B.M. Greenberg. Department of Biology, University of Waterloo, Waterloo, ON.

Phenanthrenequinone (PHQ) is a photoproduct of phenanthrene (PHE), one of the most prevalent PAHs in environment. PHQ is a compound of substantial toxicological interest because its toxicity was greatly promoted comparing with its parent PAH PHE to aquatic modern organisms such as *Vibrio fischeri*, duckweed (*Lemna minor*), *Daphnia*. The toxicity of quinones is generally thought to occur by two mechanisms: the formation of covalent bonds with biological molecules by Michael addition chemistry and the catalytic reduction of oxygen to superoxide and other reactive oxygen species (ROS) Lacking an electrophilic site, PHQ was considered to be strictly a redox cycling agent and exhibit only oxygen-dependent toxicity. Although increasing evidence suggest that ROS involves in PHQ toxicity, these data primarily come from studies using cell free systems and isolated subcellular fraction. Very rare *in vivo* evidences are available for the direct relation between ROS production and toxicity. In present paper, we examine the toxicity of PHQ to *V. fischeri* with and without O_2 and attempt to establish the mechanism of PHQ toxicity *in vivo* system. Exposing bacterial cell to PHQ, higher amount ROS and H_2O_2 than control were observed. PHQ also induced high activity of antioxidant enzyme SOD and catalase. The concentrations of PHQ that induce the toxicity, ROS and H_2O_2 production, elevated activity levels of Fe-SOD and catalase are consistent. Further examining the toxicity of PHQ under aerobic and anaerobic condition suggest PHQ possess different toxic mechanism in various concentrations. Under aerobic condition, PHQ greatly inhibit the growth of *V. fischeri* at the concentration of 200 $\mu g \cdot L^{-1}$ and completely eliminated the bacterial growth at the concentration of 300 $\mu g \cdot L^{-1}$. However, under anaerobic condition, PHQ did not show any toxic to *V. fischeri* at concentration lower than 1250 $\mu g \cdot L^{-1}$, suggesting that at low PHQ concentration (200 to 1250 $\mu g \cdot L^{-1}$), production of ROS may be the only toxic mechanism of PHQ to *V. fischeri*. At higher concentration, PHQ show toxic even under the anaerobic condition, showing another different toxic mechanism of PHQ.

Towards national agri-environmental water quality standards for waterborne pathogens. R.A. Kent[1], T.Edge[1], R. Phillips[2] and D. Klonowska[3]. [1]Environment Canada, National Water Research Institute, Gatineau, QC; [2]Environment Canada, National Water Research Institute, Burlington, ON; and [3]Department of Biology, University of Ottawa, Ottawa, ON.

The Canadian Agricultural Policy Framework (APF), initiated in 2003, includes development a suite

of non-regulatory agricultural-environmental standards to specify desired levels of environmental stewardship required of agriculture. The National Agri-Environmental Standards Initiative (NAESI) is Environment Canada's principal endeavour under the APF. Environmental quality performance standards will be developed, field-tested and recommended to Agriculture and Agrifoods Canada (AAFC) for use in supporting the most effective and environmental sustainable Best Management Practices (BMPs). To facilitate this effort, AAFC has established and funded a national network of seven experimental Watersheds Evaluation of Beneficial management practices sites (WEB-sites) designed to measure on-farm environmental performance of these management practices related to environmental themes.

The NAESI water theme has initiated research and development utilizing some of the WEB-sites for the development and testing of national agri-environmental standards for water theme components: nutrients, pesticides, water conservation and waterborne pathogens. Selection and research and development of reliable and cost-effective Indicators of Waterborne Pathogens is the focus of this work. It includes review and analysis of physical, chemical and microbial indicators currently being used or in development (particularly in Canada) in determining occurrence (detection), quantity/levels, source, and relative risks of waterborne pathogens to human and non-human receptors. The project will investigate and review existing Canadian capacity to evaluate occurrence of agriculturally-derived waterborne pathogens (bacteria, protozoa, viruses), their relative risks and their measured impacts (e.g., incidence of waterborne disease outbreaks, shellfish contamination, wildlife and livestock illness and death, crop contamination from irrigation etc.). However, the principal focus of the project is to outline and describe in detail, waterborne pathogens themselves, in particular, those posing the greatest risks to water quality and water uses in Canada. This analysis will include details of the prevalence of waterborne pathogens and indicator micro-organisms in watersheds both with and without Intensive Livestock Operations (ILO's; typically of bovine, avian or porcine origin). It will examine impacts on both human and non-human receptors (including wildlife, domestic livestock, and agricultural crops) all of which are potential vectors for waterborne pathogen transfer. This will lead to recommendations of relevant indicator suites which can then be tested in experimental watersheds for application as non-regulatory performance standard tools. These tools can then be used to guide agriculture and environmental agencies, farmers and watershed communities in sustaining "healthier water" through adherence to achievable performance standards (APS) with an eye to moving toward ideal performance standards (IPS).

This research will provide the initial impetus for pathogen standards development. It will also identify relevant knowledge gaps as well as pathogens of concern in specific watersheds or sub-watersheds with significantly unique bio-physicochemical characteristics. These characterizations will include AAFC WEBs and other relevant experimental agricultural watersheds.

Wetland mescosms for ecotoxicological studies: design and testing. E.K. Wallace[1], N.E. Glozier[1], M. Waiser[2], J.K. Holm[2] and D. Donald[1]. [1]Environment Canada, National Water Research Institute, Saskatoon, SK; and [2]Environment Canada, Ecosystem Health Assessment, Saskatoon, SK.

Many prairie wetlands located within agricultural land are susceptible to herbicide overspray. However, effects of low-level episodic exposure to herbicides mixtures are relatively unknown; due in part to the difficulty associated in assessing effects with field surveys. Mesocosms offer a method to study treatment effects as they provide environmentally realistic, statistically replicated conditions. In Saskatchewan, two systems have recently been used to assess the effects of herbicide overspray. In June 2004, an enclosure design with 2 m rigid plastic cylinders embedded 20 cm into the wetland sediment and enclosing a community of algae, zooplankton and benthic invertebrates were used to test the effects of four sulfonylurea herbicides applied singly at simulated overspray concentrations.

For all endpoints measured no significant effects relative to the control were detected. In 2005, a novel mesocosm system designed as a self-sustaining wetland food web was used at a wildlife area near St Denis. Each mesocosm system consisted of a large tank (1.8 m diameter) with five smaller replicate tanks into which sediment and invertebrates communities were randomly inoculated. A submersible pump circulated treatment water to all replicates. Herbicides tested singly and as registered tank mixtures were thifensulferon methyl, MCPA, fenoxyprop and clopyralid. Endpoints measured included density and productivity of bacteria, algae, macrophytes, zooplankton and benthic invertebrates. This novel mesocosm system maintained a functioning wetland community for over 28 d and demonstrated the benefits of using mesocosm systems to evaluate effects of contaminants.

Laurentian SETAC. N.C. Feisthauer[1] and L. Van der Vliet[2]. [1]Stantec Consulting Ltd., Guelph, ON; and [2]Environment Canada, Environmental Technology Centre, Ottawa, ON.

Laurentian SETAC is the local Ontario Chapter of the Society of Environmental Toxicology and Chemistry (SETAC), a global society that promotes the advancement and application of scientific research related to contaminants and other stressors in the environment, education in the environmental sciences, and the use of science in environmental policy and decision-making. We share the Aquatic Toxicity Workshop's commitment to involving professionals from academia, government and industry/consulting in our meetings and governance, and encourage student participation in all our activities. Laurentian SETAC science includes both terrestrial and aquatic environments, and encourages research from many levels of biological organization, from cellular to ecosystem. Activities such as short courses, dinner meeting and the annual general meeting and conference are held throughout the year in venues across Ontario, and topics reflect our interdisciplinary membership. Our diverse membership is one of Laurentian SETAC's strengths, as risk assessors, regulators, laboratory technicians, project managers, professors and students share our forums for communicating their work and networking with colleagues. Laurentian SETAC is your window into Ontario environmental toxicology and chemistry. Our website is www.LaurentianSETAC.ca.

Approaches for assessing the ecological impacts of existing substances in groundwater. S. Schnabel[1], Y. Couillard[1], N. O'Driscoll[1], M.B. Constable[2], J. Kurias[1], D. Boivin[1], D. Delage[1], D.W. Gutzman[1] and P. Doyle[1]. [1]Environment Canada, Existing Substances Branch, Gatineau, QC; and [2]Environment Canada, Toxic Substances Division, Edmonton, AB.

Under the Canadian Environmental Protection Act, 1999 (CEPA), the Ministers of the Environment and Health must conduct screening assessments on substances on the Domestic Substances List (DSL) that meet the categorization criteria for persistence and/or bioaccumulation and inherent toxicity, or for greatest potential for human exposure. A screening assessment determines whether a substance poses a risk to the Canadian environment, by examining available information and developing conclusions based a weight of evidence approach as required by the Act. In order to gain experience and develop guidance for tackling these assessments, a Screening Assessment Pilot Project has been initiated consisting of 123 DSL substances expected to meet categorization criteria. Initial screening of some substances on the pilot project shows that certain substances occur at relatively high concentrations in groundwater closely associated with sites of anthropogenic activity (e.g., industrial sites, landfills, contaminated sites). This raises the question of how to characterize the risk posed by groundwater contaminants in ecological screening assessments. Currently, the Existing Substances Branch of Environment Canada is re-evaluating its approach to assessing groundwater and groundwater contamination. This poster presents an overview of different options for science-based approaches for groundwater in ecological risk assessment. The implications of data gaps in the fate

of substances in groundwater and the lack of ecotoxicological data for groundwater organisms are considered.

Modified Japanese medaka (*Oryzias latipes*) embryo larval bioassay for rapid determination of developmental abnormalities. A.J. Farwell, V. Nero, M.V. Croft, P. Bal and D.G. Dixon. Department of Biology, University of Waterloo, Waterloo, ON.

The Japanese medaka (*Oryzias latipes*) embryo larval assay has been used extensively as a laboratory model for the evaluation of toxicant impacts. However, the naturally high variability in the time of hatch is a limitation in terms of the duration of the test. To improve the economic feasibility of this bioassay, we modified an 18 day test protocol by gently agitating the test containers to synchronize hatching and induce early hatching. For non-exposed embryos, the modified protocol with agitation resulted in median hatch times of 7 to 8 d compared to 9 to 14 d for the 18 d protocol without agitation. To determine whether agitation had any effect on the sensitivity of sublethal endpoints, the 18 d and modified protocols were compared using two different complex mixtures containing either polycyclic aromatic hydrocarbons (PAH EXT) or naphthenic acids (NA EXT). Agitation reduced time to hatch by = 1/3 and improved hatch success (100%). The modified protocol was generally more sensitive for a stressor (ie., PAH EXT) which caused reduced hatch length and induced symptoms of blue sac disease (BSD) including heart, yolk sac-pericardial, and cranial-skeletal deformities but had no effect on time to hatch. In contrast, the 18 d test was more sensitive for a stressor (i.e., NA EXT) that had no effect on hatch length, was a weak inducer of BSD symptoms but increased time to hatch. The data suggests that differences between the protocols may be a function of the chemical properties of the test compound(s) and their ability to induce BSD symptoms. Although there was variation in the sensitivity of the measurement endpoints for the modified protocol, this protocol is recommended as a cost effective and rapid method to screen the sub-lethal impact of toxicants on the early-life stages of fish.

Application of pH-controller-technology to low-hardness/low-alkalinity solutions when testing acute lethality of effluents to rainbow trout (*Oncorhynchus mykiss*). R. Chong-kit and J. Schroeder. Ontario Ministry of the Environment, Laboratory Services Branch, Etobicoke, ON.

The Ontario Ministry of the Environment (MOE) adapted pH controller technology and applied it to pH stabilization in rainbow trout acute lethality tests, conducted according to the standard Environment Canada test method. The pH controller was set at each sample's initial pH, and carbon dioxide was delivered into the test solutions as required to maintain pH. The technique was shown to maintain initial test pH within 0.1 pH unit throughout the 96 h test period in well buffered, moderately hard solutions. To determine the applicability of the controller method to low alkalinity/low hardness solutions, the MOE carried out a series of experiments using effluents and dilution waters with various alkalinities and hardnesses. The pH controller test system will be described along with the results of tests carried out in various solutions of low alkalinity and pH and the operational range of the controller will be presented

Evaluation of buffer zone effectiveness in the protection of aquatic environments in Prince Edward Island - 2004. A.M. Dunn[1], W.R. Ernst[1], M. Bernier[2], A. Cook[2], K.G. Doe[2], P.M. Jackman[2] and G. Julien[2]. [1]Environment Canada, Environmental Protection Branch, Dartmouth, NS; and [2]Environment Canada, Environmental Conservation Branch, Moncton, NB.

Runoff from potato fields has lead to pesticide induced fish kills in Prince Edward Island over the past decade. In an effort to minimize the risk posed by row crops, the PEI government introduced legislation in 2000 that stipulates a 10 m vegetated buffer zone must be maintained between row

crops and water courses on fields with slopes less than 5%. Fields with greater slopes are required to maintain a 20 m buffer. Since 2001, Environment Canada has been involved in an on-going Pesticide Science Fund supported study to test the effectiveness of these vegetative strips in reducing toxicity, nutrient and pesticide loads to surrounding aquatic ecosystems. In 2004, eleven fields were selected in PEI for runoff collection. Sample collectors were set up at the edge of the field (0 m), 10 m downslope in the buffer, and at one field at 15 m, and another field at 20 m. There were three rainfalls during the 2004 growing season that were sufficient to produce runoff at one or more of the fields. Samples were collected within 24 h of the rainfall event and analysed for pesticides, water quality parameters and assayed for acute toxicity (48 h) to *Daphnia magna*. Although the 10 m buffer zone was generally effective at reducing both field pesticide concentrations and *D. magna* toxicity, there were instances where pesticide loadings were not sufficiently reduced to eliminate *D. magna* toxicity at 10 m and these appeared to be related to runoff from tractor-compacted rows. Statistical analyses were conducted to determine if field slope, size, or meteorological conditions were correlated to pesticide concentrations and toxicity observed in test animals.

Goldfish (*Carassius auratus*) estrogen receptor subtype auto-regulation by 17β-estradiol *in vivo*. V.L. Marlatt, J. Lakoff, K. Crump, V.L. Trudeau and T.W. Moon. Department of Biology, University of Ottawa, Ottawa, ON.

In teleosts, the functional role and regulation of the recently discovered estrogen receptor (ER) subtypes (ERα, ERβ and ERγ) is largely unknown. This study investigated the regulation of the ER subtypes by 17β-estradiol (E2) in tissues within the hypothalamic-pituitary-gonad axis of male goldfish (*Carassius auratus*). Sexually mature male goldfish were continuously exposed to a nominal waterborne concentration of 1 nM E2 or solvent (0.001% ethanol) in static renewal exposures conducted for 1 and 6 days in the month of January. Plasma E2 levels increased approximately 4 to 5 fold in goldfish exposed to E2 at both day 1 and 6 compared to the solvent control treatment. A multiplex real-time RT-PCR system using TaqMan chemistry and dual-labelled fluorescent probes was developed to simultaneously measure mRNA levels of the three ER subtypes (α, β, and γ) compared to the control gene (β-actin) in testes, liver, hypothalamus and pituitary. Compared to the solvent control treated fish, waterborne exposure to E2 caused a significant increase in ERá mRNA transcripts at day 1 (20 fold) and 6 (12 fold) in liver, a significant decrease in ERβ mRNA transcripts (2 fold) in testes, while no effects were observed in any other tissues. These results support the notion that ERα may play a significant role during vitellogenesis in the liver. However, the significance of decreased ERβ in the testes and the lack of auto-regulation of the ER subtypes in hypothalamus and pituitary at a physiologically relevant level of E2 has yet to be elucidated.

Mercury levels in commercial freshwater fish species from China. W.W.M. Chan, L.M. Campbell, Y.X. Wang and B. Murray. Department of Biology, Queen's University, Kingston, ON.

Mercury contamination is a necessary area of study in China, where diets rely greatly on fish; yet little is known about Hg levels in freshwater fish. In May 2005, two species of catfish (*Silurus meridionalis* and *Mystus nemurus*) and two species of carp (*Aristichthys nobilis* and *Ctenopharyngoden idellus*) were sampled at local fish markets near 4 Chinese lakes: Tai Lake and Dongting Lake, which are large, shallow, highly industrialized water bodies; Qiandao Lake, a deep man-made reservoir, and Xinshan Lake, a smaller lake south-west of TL. The food web structure formed using stable isotope data from Tai Lake and Qiandao Lake confirmed trophic relationships between fish species and provided evidence of Hg biomagnification. Overall, mean total Hg concentrations, in $mg \cdot kg^{-1}$, were observed in the following order: Qiandao Lake (0.668) > Xinshan Lake (0.615) > Dongting Lake (0.173) > Tai Lake (0.071). Surprisingly, total Hg in fish from DTL

and TL appears to be below consumption limits of China's *Food Hygiene Law* (0.3 $mg \cdot kg^{-1}$) but higher in fish from Qiandao Lake and Xinshan Lake. Thus, evidence exists that industrialization is not the main source of Hg in these lakes, and the lack thereof is not an indicator of environmental health. High total Hg concentrations in Qiandao Lake fish is a cause for concern since many commercial fish are farmed there. These results provide baseline data for future investigations.

Cadmium affects F-Aactin cytoskeletal structure in a human hepatocellular (HepG2) cell line: preliminary findings. L. Hornung, E. Weinhofer and P.F. Dehn. Department of Biology, Canisius College, Buffalo, NY.

Lysophosphatidic acid (LPA) alters cytoskeletal structure, as does Cd, an environmental contaminant, whose mechanism of toxicity is unknown. The purpose of this study was to confirm LPA induction of F-actin polymerization in HepG2 cells and then examine cadmium's impact on F-actin using fluorescent microscopy. HepG2 cells were exposed to LPA or to LPA and then cadmium for 2 or 8 h. F-actin fluorescent intensities (FAI's) were higher in LPA treated cells (104.6 ± 93.4) when compared to untreated cells (72.7 ± 16.3); indicating LPA induction of F-actin. LPA treated cells contained a higher percentage of cytoplasmic actin bundles (CAB's), a mesh-like cytoplasm (MESH), a thick F-actin band (TB's) and cable-like structures (Cables) when compared to untreated cells (72.5%, 100%, 80%, 57.5% vs. 45%, 87.5%, 77.5%, 2.5%, respectively). No significant changes in FAI's occurred between Cd treated and untreated cells, which indicated F-actin had not depolymerized. However, changes in cytoskeletal structure did occur. Both 2 and 8 h Cd exposed cells showed a decrease in retraction fibers, CAB's, MESH, and an increase in Cables when compared to untreated cells (2 h: 32.5%, 60%, 75%, 55% vs. 80%, 80%, 95%, 20%, respectively: 8 h: 12.5%, 30%, 60%, 65% vs. 60%, 75%, 97.5%, 45%, respectively). The 8 h Cd treated cells also showed a decrease in microspikes and TB's when compared to untreated (17.5%, 55% vs. 50%, 65%, respectively). These changes in cytoskeletal structure may prevent cell migration and repair, altering cell function, leading to toxicity.

Preliminary spatial and temporal trends in tissue mercury burdens in two fish species in southeastern Ontario fish over two 7-year periods (1976 to 1982 and 1997 to 2003). E. De Long[1], L.M. Campbell[1], G. Mierle[2], W. Schneider[3] and A. Hayton[3]. [1]Department of Biology, Queen's University, Kingston, ON; [2]Ontario Ministry of the Environment, Environmental Monitoring and Reporting Branch, Dorset, ON; and [3]Ontario Ministry of the Environment, Environmental Monitoring and Reporting Branch, Etobicoke, ON.

Tissue Hg concentrations in many fish species, particularly those in higher trophic levels, are elevated in lakes and rivers across Ontario, Canada. It is widely believed that Hg burdens in fish first started to rise at the time of the Second World War, and that they reached a maximum during the late 1960s and early 1970s. The Ontario Ministry of Environment (OME) has been collecting data on fish tissue Hg burdens in lakes and rivers across Ontario since the mid-1970s to the present. It is estimated that 165,000 to 170,000 individual fish, from 86 species and 1600+ sites, have been tested for Hg, with this equating to about 1.5 million database records across Ontario. While Hg burdens in fish have reportedly been declining since the mid-1970s, burdens in many species still often exceed Health Canada guidelines for human consumption. While the OME uses these data primarily for the production of the biennial Guide to Eating Ontario Sportfish and for the identification of Hg sources, we have used a geographic information system (GIS) to quantify the spatial and temporal distribution trends for Hg burdens in Southeastern Ontario in two species at different trophic levels: lake trout (*Salvelinus namaycush*) and yellow perch (*Perca flavescens*). We will present our GIS mapping results and discuss the relevance of the spatial / temporal trends.

Terrestrial ecotoxicity/Écotoxicité terrestre

Session chairs/Présidents: Natalie Feisthauer and/et Juliska Princz

Assessment of metals in soil: influence of confounding variables. G.L. Stephenson[1], N.C. Feisthauser[1], J.I. Princz[2], M-K. Gilbertson[3], J. Roembke[4] and S. Jänsch[4]. [1]Stantec Consulting Ltd., Guelph, ON; [2]Environment Canada, Environmental Technology Centre, Ottawa, ON; [3]C. Wren and Associates, Guelph, ON; and [4]ETC Oekotoxikologie GmbH, Florsheim, Germany.

Toxicity assessments of soils contaminated with metals or metal mixtures frequently include an earthworm reproduction test because it is considered to be sensitive and ecologically relevant. The test usually comprises one component of a test battery for site assessments, ecological risk assessments, and for assessing the toxicity of a negative control soil that has been amended with a particular metal or metal mixture. The negative control soil can be either a formulated artificial soil or an uncontaminated, field-collected reference soil. Reference soils are soils with physico-chemical characteristics that are similar to those of the contaminated site soil but free of the contaminant(s) of interest. For an ecotoxicity assessment of an environmentally contaminated, field-collected site soil, the physico-chemical characteristics of the soil itself can significantly influence the response of the test organism, independent of, or in synergy with, the effects of the contaminant(s). Therefore, selection of an appropriate experimental control soil should include provision of a measure of the matrix effects on the test organism. Results from earthworm reproduction tests with eleven Canadian and eight German reference soils were compared to those with an artificial soil to determine the implications of the use of field-collected reference control soils in terrestrial toxicity testing. Several case studies will be discussed to demonstrate that, for toxicity assessment of contaminated site soils, it is critical to include whenever possible a reference control soil.

Terrestrial toxicity testing of metal contaminated soils from Sudbury, Ontario. L. Van der Vilet[1], J.I. Princz[1], M-K. Gilbertson[2] and R.P. Scroggins[1]. [1]Environment Canada, Environmental Technology Centre, Ottawa, ON; and [2]C. Wren and Associates, Guelph, ON.

An ecological risk assessment (ERA) in Sudbury will examine and evaluate the possible risks associated with airborne metal particulate emissions resulting from past smelting operations. As a result, terrestrial toxicity testing was undertaken to support decision-making for this ERA. Soil was collected from a reference site with a low background concentration of metals and from a site with a high concentration of metals; both soils were acidic. Survival, growth, and reproduction of the test species (*Folsomia candida*, *Eisenia andrei*, red clover, and northern wheatgrass) were assessed, both with and without partial pH neutralization, to examine the influence of low soil pH on toxicity. *F. candida* were not sensitive to soil acidity or to metal contamination levels. *E. andrei* were highly sensitive to soil pH and metal contamination. Decreased reproduction was evident in the unadjusted reference soil, and in the contaminated soil. Red clover (*Trifolium pratense*)and northern wheatgrass (*Elymus lanceolatus*) were sensitive to low pH and metal contamination levels, and root growth proved to be the most sensitive endpoint. Both plants showed increased growth in soils that had been pH adjusted, and increased growth in reference soil when compared to metal contaminated soil. To focus future testing of other metal contaminated sites, we recommend the continued use of a battery of test organisms to capture the varying sensitivity of test organisms.

Multiple lines of evidence collected to assess the impact of metals in forested areas of Sudbury. M-K. Gilbertson and C.D. Wren. C. Wren and Associates, Guelph, ON.

The Ecological Risk Assessment (ERA) in Sudbury will evaluate the possible risk from airborne particulate emissions from the chemicals of concern (COCs) which are Ni, Cu, Co, As, Pb and Se.

An area of uncertainty in the ERA is whether the soils in the Sudbury area are inhibiting the recovery of a self-sustaining forest ecosystem. The overall aim of the field and laboratory studies conducted in 2004 and 2005 was to determine whether the concentrations of the COCs present in the Sudbury soils pose an unacceptable risk to plants, invertebrates or microbial activity. This risk will be determined using a battery of single species terrestrial toxicity tests, field-based ecological measurements and soil chemistry. The results will be integrated to determine whether the concentrations of metals in the soil are inhibiting the recovery of a self-sustaining forest system.

Development of terrestrial plant toxicity methods using species representative of Canadian boreal forests. M. Moody[1] and R.P. Scroggins[2]. [1]Saskatchewan Research Council, Environment and Minerals Branch, Saskatoon, SK; and [2]Environment Canada, Environmental Technology Centre, Ottawa, ON.

The need for a standardized method using boreal forest plants suitable for testing of contaminated soil has been recognized by both industry and Environment Canada.Since Environment Canada's workshop "Toxicity Test Methodologies for Assessing the Impacts of Contaminant Mixtures in Soil Using Terrestrial Species of Ecological Relevance in Canadian Soil Systems" (February, 2003), four projects have been completed. A survey of boreal forest plants across Canada highlighted 219 species. This list was refined by criteria including abundance in the boreal forest and in Canada, reproduction from seed, size of seedling and recommendations from five ecologists. Seven species have undergone testing to determine germination and stratification techniques, and typical growth in artificial soil. Natural boreal forest soils of a variety of types and textures were collected and the growth of six plant species used to recommend suitable test durations. The successful species include white spruce (*Picea glauca*), black spruce (*Picea mariana*), jack pine (*Pinus banksiana*), goldenrod (*Solidago sp.*), bluejoint reedgrass (*Calamagrostis canadensis*) and trembling aspen (*Populus tremuloides*) although additional species are under consideration. Comparison of these plants using reference toxicant tests using boric acid is presented. Further applications of the test include the use of white spruce and goldenrod to evaluate metal contaminated soils.

Biotransformation of the polycyclic nitramine CL-20 by glutathione S-transferase. G. Bardai[1], J. Hawari[2], P.A. Spear[3], J. Hoang[1], S. Grosse[4] and G.I. Sunahara[1]. [1]National Research Council of Canada, Applied Ecotoxicology, Montreal, QC; [2]National Research Council of Canada, Environmental Chemistry, Montreal, QC; [3]Department of Biological Sciences, University of Quebec at Montreal, Montreal, QC; and [4]National Research Council of Canada, Chemistry, Montreal, QC.

Hexanitrohexaazaisowurtzitane (CL-20), a highly energetic polycylic nitramine is an emerging environmental contaminant. Our earlier studies showed that adult quail exposed to CL-20 led to increased liver weight and liver aspartate aminotransferase activities, and yet CL-20 was not detectable in quail brain, heart, spleen, or liver. The possibility that adult quail liver may biotransform CL-20 was considered. Liver homogenates were prepared from adult female Japanese quail (*Coturnix coturnix japonica*) prior to the CL-20 biotransformation assays and the glutathione S-transferase (GST) purification procedures. Data indicates that the disappearance of CL-20 using whole cytosol was inhibited in vitro by either ethacrynic acid or the glutathione (GSH) analogue, s-octylglutathione, and suggested the involvement of GST. Using size exclusion, and affinity chromatography, we purified and characterized a cytosolic GST from quail liver capable of biotransforming CL-20. The molecular masses of the purified proteins were about 28 and 27 kDA. Partial N-terminal sequence analysis showed both alpha and mu classes of GST, having 100% homology to chicken and quail GST described in the literature. For comparative purposes, we also purified a rabbit GST enzyme. With the requirement of GSH, purified enzyme preparations from quail and rabbit livers

biotransformed CL-20 at the rates of 0.27 ± 0.05 and 0.38 ± 0.02 $nmol^{-1}min \cdot mg^{-1}$ protein, respectively. Data suggest that the purified protein can biotransform CL-20, as evidenced by the concomitant formation of nitrite (NO2-) with CL-20 removal. These data suggest that GST purified from female quail liver is capable of biotransforming CL-20 *in-vitro*, and may explain the absence of CL-20 in liver *in-vivo*.

Validation of Environment Canada biological test methods for assessing contaminated soils: plant toxicity tests. J.I. Princz and R.P. Scroggins. Environment Canada, Environmental Technology Centre, Ottawa, ON.

Environment Canada is continuing its efforts to develop, validate, and publish standardized toxicology methods for the testing of field-contaminated and substance-amended soils. Standardized toxicity test methods have been developed using species representative of terrestrial invertebrates (e.g., earthworms and arthropods) and plants inhabiting soil ecosystems in Canada. Prior to publication, each soil toxicity method must be peer-reviewed by experts and validated through inter-laboratory testing. In preparation for the publication of plant toxicity test methods, Environment Canada co-ordinated a series of inter-laboratory studies involving six laboratories across Canada. The plant studies included an assessment of a 7 d reference toxicant test using artificial soil amended with boric acid, as well as a 14 d definitive test using amended soil, as well as the dilution of a field-collected contaminated soil. The mean 7 d IC50 for shoot length, the required endpoint for reference toxicity tests, was 726 mg $H_3BO_3 \cdot kg^{-1}$ soil dry wt., with values for individual laboratories ranging from 379 to 961 $mg \cdot kg^{-1}$. The co-efficient of variation was 28%, an acceptable level of between-laboratory precision. The co-efficient of variation for all four endpoints for the 14 d definitive test using boric acid and artificial soil ranged from 7 to 22%, indicative of good laboratory precision. The results obtained from each of the plant test options will be summarized and discussed with regards to the observed within and between-laboratory
variability.

The use of control performance data for QA/QC of chronic plant toxicity tests. K.M. Olaveson, G.L. Stephenson, N.C. Feisthauser and J.T. Crumb. Stantec Consulting Ltd., Guelph, ON.

Current toxicity test methods in terrestrial ecotoxicology recommend the use of reference toxicant tests to determine if changes are occurring in laboratory culture test organism sensitivity to a standard reference chemical. A change in sensitivity might affect their acceptability for use in toxicity testing. Reference toxicity tests involve significant time to set up, monitor, process, and analyze the data. They are either conducted on a regular basis or are conducted concurrently with a toxicity test. These tests require significant space (e.g., growth chambers) and materials in order to be conducted. This testing is in addition to regular toxicity testing that occurs in a laboratory. Also, in the case of plant testing, many different species are used, and they are often used on an irregular basis, depending on the requirements of particular projects. We have investigated the use of control performance data as an alternative QA/QC check for laboratory toxicity testing. The results from the experimental control treatment from tests conducted in our laboratory were combined into a warning chart for comparison of control performance over time. The benefit of using the control performance data is that the time, space and materials spent producing QA/QC data are reduced. Similar to reference toxicity testing, the use of control performance data allows for comparisons of test results over time. It is important to ensure that all data being used in the comparison of control performance data were generated under the same conditions (e.g., same soil, test duration, test conditions, and endpoints) because differences in any of these test characteristics could influence the response of the test organisms in the control treatment. Control performance charts for toxicity tests conducted with artificial soil, which is a fine

sandy loam soil formulated from constituents, were produced for plant species including: barley (*Hordeum vulgare*), alsike clover (*Trifolium hydridum*), perennial ryegrass (*Colium perenne*), red fescue (*Festuca ruba*), northern wheatgrass (*Elymus lanceolatus*) and red clover (*Trifolium pratense*) (shoot length, root length, shoot dry mass, and root dry mass). The performance control charts indicate the variability associated with measurement endpoints for tests repeated over time and are a useful QA/QC tool.

Ecotoxicity assessment of petroleum hydrocarbon residuals in site soils and paired reference soils. J.T. Crumb[1], N.C. Feisthauser[1], G.L. Stephenson[1], M. Tindal[2] and C. Meloche[3]. [1]Stantec Consulting Ltd., Guelph, ON; [2]Axiom Environmental Inc., Calgary, AB; and [3]Husky Energy Ltd., Calgary, AB.

An ecotoxicity assessment was conducted with the earthworm *Eisenia andrei* and 12 site soils contaminated with weathered and aged petroleum hydrocarbon (PHC) residuals. The site soils were matched with reference soils that had similar physico-chemical characteristics but were free of contamination. The chronic screening tests were 63 d in duration and the measurement endpoints included adult survival at the end of 14 and 35 d, as well as, progeny production at the end of the test. The tests were conducted according to Environment Canada's biological test method for earthworm survival, growth, and reproduction. No adverse effects were observed for survival of adult earthworms on day 14. Significant adult mortality was observed for only one of the 12 site soils on day 35. There were no clear trends with respect to the effects of PHC residuals on progeny production. Reproduction was significantly greater in three of the reference soils relative to their paired site soils. Reproduction was significantly lower in two reference soils relative to the paired site soils and, for five of the site soils, there were no significant differences in progeny production relative to the paired reference soils. Close scrutiny of the results of these tests and the physico-chemical characteristics of the soils indicated that some of the reference soils were poorly matched with the site soils and that some of the soils (reference and site soils) were sub-optimal for growth and reproduction of the test species. Earthworm reproduction is often poor in soils with the combination of low organic matter and textures that are very clayey or very sandy. The results of this study emphasize the importance of understanding the effects of soil characteristics on the test organisms independent of the effects on the contamination.

Use of a soil conditioner with sub-surface and/or sub-optimal contaminated site soils for earthworm toxicity testing. N.C. Feisthauser[1], J.T. Crumb[1], K.M. Olaveson[1], G.L. Stephenson[1], M. Tindal[2] and C. Meloche[3]. [1]Stantec Consulting Ltd., Guelph, ON; [2]Axiom Environmental Inc., Calgary, AB; and [3]Husky Energy Ltd., Calgary, AB.

Earthworm reproduction is a sensitive and ecologically relevant assessment endpoint often used to evaluate the site-specific risks associated with contaminated soil. Because of their short life cycle, ease of culture and prolific reproductive capability, the most commonly tested earthworm species are *Eisenia andrei* and *E. fetida*. Both are compost worms, and their natural habitat is moist, highly organic soils. However, many contaminated site soils have physicochemical characteristics that constitute suboptimal habitat for growth and reproduction of earthworms. In particular, soils contaminated with petroleum hydrocarbons as a result of oil drilling activities are often sub-surface soils with little organic matter. Earthworm reproduction is often poor in soils with low organic matter, whether contaminated or not. If acceptable reproduction does not occur in reference soils, then it is not possible to determine the effects on reproduction of contaminants in a corresponding contaminated soil. *E. andrei* reproduction was evaluated in different uncontaminated reference and petroleum hydrocarbon (PHC)-contaminated site soils amended with a soil conditioner that would decrease the

bulk density of the soil and provide more organic matter but would not otherwise change the texture or other physicochemical characteristics of test soils in order to test the hypothesis that earthworm reproduction would be improved in these soils. The soil conditioner was buffered ($CaCO_3$) sphagnum peat. It is well tolerated by earthworms and is relatively inert since earthworms rarely use it as a food source. One pair of reference and contaminated soils was amended with 0, 1.25, 2.5, 5 and 10% peat. Total and bioavailable concentrations of petroleum hydrocarbons were determined in amended and non-amended soils. Significant increases in earthworm reproduction occurred in the reference and contaminated soils at the 2.5% peat treatment, relative to reproduction in the 0% treatments. Following this test, three other pairs of reference and PHC-contaminated soils were amended with 2.5% peat to evaluate the efficacy of the soil conditioner with different soil types. The effect of the peat amendment on PHC bioavailability was also measured. Preliminary test results suggest that amending site soils that are suboptimal for earthworm reproduction with buffered peat is an effective method to discriminate effects on earthworm reproduction that are attributable to soil physicochemical characteristics versus contaminants in soil.

Contributions of riparian vegetation to mercury cycling in shield lakes. E.I. Siwik[1], G. Mierle[2], N. Knezevich[2] and L.M. Campbell[1]. [1]Department of Biology, Queen's University, Kingston, ON; and [2]Ontario Ministry of the Environment, Environmental Monitoring and Reporting Branch, Dorset, ON.

Much of the Hg research in Ontario has centered on aquatic environments since fish can be a significant source of methyl-mercury for humans. Terrestrial environments and their interactions with aquatic environments in regards to the Hg cycle have received little attention. Litterfall from trees in riparian areas has been identified as an important source of carbon to Ontario aquatic environments but little work has been done on other elements. We are now focusing on the role of trees as a source of Hg in aquatic environments in the Muskoka-Haliburton region of central Ontario. Leaves are being collected from trees along lakes (e.g., Harp, Dickie) that are included in the Ontario Ministry of the Environment's calibrated watershed project. Preliminary studies conducted by the Ontario Ministry of the Environment regarding spatial and temporal variation of Hg content in leaves collected in Dorset, Ontario, have found no significant difference in Hg content between canopy leaves and those from a height of 2 m, or from the north or south sides of the tree. There was however, a significant increase in Hg content in leaves over a period of two summer months. Following these evaluations of Hg distribution in trees, detailed studies and models focusing on the link between terrestrial vegetation and the aquatic Hg cycle will be undertaken.

Ecotoxicological risk assessment (ERA) for total petroleum hydrocarbons (TPH): new developments. A-M.A. Lafortune[1], L. Martel[1] and R. Chassé[1], C. Bastien[2] and R. Gauthier[2]. [1]Ministère du Développement durable, de l'Environnement et des Parcs, Centre d'expertise en analyse environnementale du Québec, Sainte-Foy, Qc; et [2]Ministère du Développement durable, de l'Environnement et des Parcs, Service des lieux contaminés, Sainte-Foy, Qc.

As part of a research strategy supporting the Ministère du Développement durable, de l'Environnement et des Parcs Soil Protection and Contaminated Sites Rehabilitation Policy and the corresponding rules, an ecotoxicological reference values (ERVs) guideline protocol has been developed. The goal of this protocol was to allow, in a systematic and coherent way, the generation of new ERVs suited to the Policy management goals based on an exhaustive review of the literature. This project generated new ERVs for fifty substances, including metals and organic compounds. However, it was not possible to generate ERVs for TPH due to the paucity of the toxicity database. This represents a major limitation since the majority of Québec's contaminated sites involve petroleum mixtures. To respond to our risk evaluation and management needs, two interdependent

research projects were thus begun. The objectives of the first project are: generate ERVs for crude oil; develop an adapted toxicity tests battery for petroleum hydrocarbons (PHC); validate the generated ERVs while comparing the application of two analytical methods (one based on fractions, the other based on total PHC) through the adapted test battery with recent and old contamination. On these bases, the second project objective is to operationalized the ERA for the PHC contaminated sites. The main aspects of the development of an adapted test battery for petroleum hydrocarbons will be presented. Firstly, each stage of the development protocol will be described: literature review, selection of relevant studies, selection of the sensitive plants and soil dwelling invertebrates, development of a more representative artificial soil, and adaptation of short and long term usual toxicity tests to these sensitive receptors. Secondly, the final test battery proposed to evaluate the effects of petroleum hydrocarbons will presented. Finally, the preliminary results of the application of this test battery to a specific petroleum mixture, fuel oil (low viscosity mazout#6), will be discussed.

Ecotoxicological risk assessment (ERA) of contaminated sites in Québec. A-M.A. Lafortune, L. Martel and R. Chassé. Ministère du Développement durable, de l'Environnement et des Parcs, Centre d'expertise en analyse environnementale du Québec, Sainte-Foy, Qc.

In the province of Québec, the management of contaminated sites involve a risk-based approach since 1998. The Soil Protection and Rehabilitation of Contaminated Sites Policy and the corresponding rules and the ERA guidelines for contaminated sites dictate the application requirements for sound scientific risk-based remediation. Thus, after having introduced the context of ERA of contaminated sites in Québec, this presentation will firstly present the important concepts and steps for the application of the ERA guidelines for contaminated sites. Secondly, it will focus on four essential, though often neglected, elements that are critical to the usefulness and credibility of ERA: data quality objectives definition, uncertainty analysis, quality assurance and control, and risk communication. Finally, a discussion on how the ERA and the environmental risk-based management could be improved based on our past experiences will be presented. Particularly, small or less complex sites, as those often presents in urban areas, typically lack sufficient resources to do site-specific ERA. For that reason, these type of sites frequently leads to confrontation between regulated and regulating risk managers, compromising the utility of ERA for the decision making process. To facilitate the application of ERA for those sites, different strategies are investigated, i.e.: exclusion criteria, checklists ERA, generic risk assessment, with generics conceptual model and assessment endpoints, and the application of specific toxicity tests battery.

Data quality objectives for aquatic toxicology/Objectifs de qualité des données pour la toxicologie aquatique

Session chairs/Président: John Purdy and/et Roxana Roshon

Key data quality elements for aquatic toxicity studies. G. Gilron[1], R.L. Breton[2] and R. Thompson[2]. [1]Golder Associates Ltd., Mississauga, ON; and [2]Cantox Environmental Inc., Ottawa, ON.

Results from aquatic toxicity studies are commonly used in the assessment of ecological risk in support of regulatory notifications of new chemicals, polymers and pesticides. Over the past two decades, a number of major initiatives have been established that have aided in the improvement of data quality in these studies. These initiatives include: (i) publication of, and regulatory requirement for, the use of standardized testing methods; (ii) accreditation of laboratories according to ISO Guide 17025; and, (iii) regulatory requirements for conducting studies consistent with Good Laboratory

Practice principles. We recently conducted a comprehensive review of a large database of over 600 acute and chronic ecotoxicity testing studies using fish, Daphnia and algae as test organisms. The original objective of this review was to develop scoring methodologies for use in assessing the quality and usability of these data, and to assess the appropriateness of the data for use in ecological risk assessments and Quantitative Structure-Activity Relationships. Based on an assessment of the ecotoxicity studies in comparison with regulatory guidance, data available from the literature, and international quality standards, we have identified a number of key elements that need to be addressed to assure the quality of these studies. These elements can be categorized using the following headings: (i) substance and test species characterization, experimental design; (ii) compliance with test validity criteria; (iii) proper test documentation; (iv) statistical treatment of data; (v) quality system monitoring; and (vi)data reporting. This presentation will elaborate on elements and provide specific rationale for their inclusion as key elements in toxicity testing study reports.

Statistical treatment of toxicology data and study design. J.C. Hall. Department of Environmental Biology, University of Guelph, Guelph, ON.

In toxicology, as well as other environmental sciences, experimental design greatly influences the interpretation of the data. Immunoassays are used as a rapid screening tool for pesticide toxicology. Examples of varied immunoassay microplate design will be presented, which influenced the outcome. The statistical treatment of the data affects the results.

Quality data = standardized methods + laboratory accreditation. L.N. Taylor and R.P. Scroggins. Environment Canada, Environmental Technology Centre, Ottawa, ON.

To ensure the generation of high quality test data by Canadian toxicological testing laboratories, the utilization of standardized testing methodologies is only half of the equation. The other half requires the assessment of laboratories' policies, procedures and practices associated with the generation of toxicological data. Over the past 15 years, the Method Development and Applications Section, Biological Methods Division at Environment Canada has produced 18 biological method documents for aquatic, sediment and soil testing applications. Each method requires approximately 6 to 10 plus years for the development, validation and standardization process. The considerable investment in time and resources in producing a standard method is matched by a strong commitment to ensuring high quality test results. In support of the Canadian Association for Environmental Analytical Laboratories (CAEAL) accreditation program for aquatic, sediment and soil toxicology methods, the Biological Methods Division has prepared detailed laboratory inspector checklists for the 18 test methods. The detailed checklists highlight the "must" and "should" requirements of the methodology. In addition, EC conducts technical training sessions for CAEAL assessors leading to more thorough laboratory inspections and reducing the variability of acute lethality and sub-lethal toxicity test results.

Aquatic toxicity testing in environmental compliance and litigation. J.N. Bishop. Stantec Consulting Ltd., Mississauga, ON.

This presentation is part of the session on Data Quality Objectives in Aquatic Toxicology. In this context, the paper examines the role of aquatic toxicity in environmental compliance and litigation, and how scientific data of any kind is used in compliance or litigation. It also examines changes in Ontario's legislation as a result of Bill 133.

Environmental Compliance in Ontario (Water) includes the following elements: (i) Federal legislation –*Canadian Environmental Protection Act* (CEPA) and the *Fisheries Act* (FA) and (ii) Provincial legislation – Ontario's *Environmental Protection Act* (EPA) and *Ontario Water Resources*

Act (OWRA). The intent of these Acts is to prohibit "persons", i.e., individuals, municipalities, companies or anyone else, from introducing deleterious substances to our waterways, or from allowing the discharge of substances that "may impair" the water, or which may have an adverse effect.

Most of us would probably agree that these rules are good things. They are, but for Defense and Crown lawyers, the words of the Acts and the scintilla of the data used to lay charges under the Acts are viewed and handled differently than scientists would. The defense lawyer will look for every possible advantage in the wording of the law or the facts behind the charges, while the Crown lawyers do their best to interpret the same language in ways that overcome the tactics of the Defense. In recent months in Ontario, the government lawyers have even gone a step further, with Bill 133, which became law in June of this year. The government lawyers have now reworded parts of the OWRA so as to make it much more difficult to use scientifically sound approaches to demonstrate that impairment may not have occurred. For example, to determine whether a release may impair water quality, the Ontario courts have decided that the substance or spill in question must actually be harmful by its nature and that the circumstances of the alleged impairment must also be proven - the impairing substance must be demonstrated to have been present in the receiving water as a result of the release, and present at a concentration and for a long enough time (duration) to have had an effect. These arguments are now not available in some cases, thanks to Bill 133, which will be discussed later in this presentation.

The point is the job of the lawyers on both sides is to find advantages for their clients wherever they can, starting with the law itself and including the scientific and other information collected as part of the investigation. Often the lawyers will rely on technical people to assist them, especially in cases where the results of specialty testing such as toxicity is involved. An example of how the same data can be interpreted differently is seen in the following hypothetical case. If 6 of 10 rainbow trout (*Oncorhynchus mykiss*) die (i.e., a "fail") when exposed to an effluent sample, statistically speaking, there's only a 68.5% chance that the test outcome of six deaths is different from five dead fish in the standard rainbow trout test (Hamilton, ATW Proceedings, 1998). If the same effluent sample had been tested 200 times, it would have passed the trout toxicity test 63 times. In an environmental prosecution where the required proof is "beyond a reasonable doubt" it's unlikely that a judge would consider 68.5% an adequate degree of certainty. Of course, enforcement officers and government lawyers generally know this and would not go trial armed only with a single aquatic toxicity result.

In my experience, toxicity testing by a properly accredited lab with demonstrable QA/QC documentation can be an important tool in defending against charges or in negotiating a favourable settlement, but is seldom the pivotal factor in successful Ontario prosecutions. For routine toxicity testing, where there is a 32% probability that the result of "fail" is no different from a "pass", one obvious solution would be to test more fish or *Daphnia*, or as has been suggested by others, base the pass/fail decision on more than six fish dying. The latter approach of applying a higher LC value might mean the Pass/Fail would be based on eight deaths. In this case, we'd be just over 90% certain that the outcome was really a fail. However, 90% certainty is not likely considered to be "beyond reasonable doubt". Neither is 99% certainty, which requires that all ten trout die. The use of more test organisms is seen in a recent suggestion that duplicate toxicity samples be taken. In this case, when toxicity samples are collected, duplicate samples would be taken and archived. If the first sample shows signs of toxicity in the first two days, and the early prognosis is they would be in the ambiguous zone of 5, 6 or 7 dead fish (or 6, 7 or 8 *Daphnia*), toxicity testing is done on the second sample. If the first result indicated six dead fish and the second showed six or more, the likelihood of someone accepting the result as a fail would be greatly increased. Is anyone likely to take this precaution, of sending the lab two duplicate sets of toxicity samples? It is not likely yet – but it could

become more likely as the implications of Bill 133 become clearer.

Bill 133, also called the "*Environmental Enforcement Statutes Law Amendment Act, 2005*", was passed on June 13, 2005. This bill applies to direct dischargers only, at least for now. Originally intended to cut down the number of spills and other polluting releases, however, it went much further, and made wide-ranging changes to Ontario's *Environmental Protection Act* and the *Ontario Water Resources Act*. Discussion reviews Ontario *EPA*, Sn 14, includes "causes or *is likely to* cause an adverse effect" becomes "causes or may cause an adverse effect". Bill 133 replaces "likely to" with "may". Most of you would agree that "may cause" is a lower standard then "likely to cause", and will result in many more releases being prohibited.

Examine Bill 133's effect on the *OWRA*, Sn 30. This section of *OWRA* prohibits the release of material if that release impairs or may impair the quality of water in a watercourse. Ontario courts had decided that in order to determine whether a release may impair water quality, both the *nature* and the material released, and the *circumstances* of the release must be assessed. That is, the material must be proven to have been present and capable of causing impairment, which means the quantity and concentration of the release as well as the time frame over which the release occurred must be considered, as well as the "form" of the material.

Bill 133 greatly expands the types of releases which are deemed to impair water quality and which are therefore prohibited by Section 30 of *OWRA*. The new deemed impairment provision is very broad. Under Bill 133, releases which are now deemed to impair water quality and are therefore prohibited include, for example, any release of a material that causes injury to or interference with aquatic organisms, according to scientific publications. How reliable is this information? Toronto Star, September 11, 2005 "Right or Wrong? It's Science" concluded that at least 50% of peer reviewed papers have incorrect conclusions. Material which could potentially "interfere" with an aquatic organism include solid materials such as sand or soil, or liquid materials such as milk orange juice.

This new provision deems the release of any such material to be impairing even though for the particular circumstances of the release, there is no potential to impair water quality. Conceivably, this provision could deem a release of a single sand or soil particle or of a drop of milk or coffee into a large lake or other watercourse to be impairing and therefore unlawful and prohibited. This is clearly ridiculous and, undoubtedly, not the intent of the new deemed impairment provision of Bill 133. However, because the provision is new and unclear, it will require considerable interpretation. It is hoped that interpretation will be reasonable, but in the meantime, there will be considerable uncertainty concerning what releases are deemed to impair water quality and are therefore prohibited by Section 30 of the OWRA.

The other major concern with Bill 133 is that it was specifically designed to allow MOE to impose environmental penalties (EPs) on companies. EPs have the following characteristics: (i) EPs are imposed within days of a spill; and (ii) the company has the onus of proving that an unlawful release did not occur ("reverse onus"). Compare this to the situation in a prosecution, where the Crown must prove its case where: (i) the burden of proof lies with the Crown; and (ii) no due diligence defense for EPs ("absolute liability"). Before Bill 133, if a company was complying with an Order from MOE issued under the *EPA*, the company couldn't be convicted in any prosecution relating to matters dealt with in the Order. Under Bill 133, this is no longer the case for an EP. Even if an MOE Director imposed an EP for a particular contravention, the company can still be prosecuted for that same offence ("double jeopardy"). The EPs now carry a price tag of $100,000 for each day the contravention occurred or continued. For this reason Bill 133 has been labelled a "tax grab" in some quarters. The net result is MOE appears to have a greater incentive to issue an EP than to go to the expense and trouble of a prosecution.

It remains to be seen whether sound science, including toxicity testing, will have a role after

Bill 133. If the government applies the changes in a reasonable way, toxicity testing and concepts such as site specific risk assessments for water quality issues could become more valuable for government and industry in achieving their goals of a cleaner, healthier environment.

Data quality in Canadian Association for Environmental Analytical Laboratories (CAEAL) accredited facilities. K. Middlebrook. Canadian Association for Environmental Analytical Laboratories, Ottawa, ON.

The Canadian Association for Environmental Analytical Laboratories (CAEAL) was established as a non-profit organization in 1989 as a result of efforts by government and private-sector laboratories to establish a mechanism to deliver quality assurance programs. The CAEAL mission is "To help laboratories achieve and demonstrate the highest levels of scientific and management excellence through the combined principles of Competence, Consistency, Credibility and Communication". The member laboratories voluntarily participate in rigorous programs of proficiency testing and accreditation, demonstrating their commitment to generate high quality data. Data quality is extremely important in analytical chemistry, and toxicology because the data can be used for regulatory purposes. Through the use of regular test specific audits and Performance Evaluation (samples CAEAL ensures that the data generated by laboratories is reliable and reproducible. Once every two years, multiple auditors with background similar to the labs review the laboratory procedures and practices. A few of the problems with data quality that are routinely encountered during audits will be discussed. There are several significant changes scheduled for the CAEAL Proficiency Testing (PT) program. These have been due to a number of factors including feedback from members during the two PT workshops conducted in 2004 and new contracts with CAEAL's collaborator laboratories. The changes, and their implementation dates, will be summarized.

Trend monitoring and data analysis/Survillance des tendances et analyse de données

Session chairs/Présidents: Michael Paine and/et Keith Somers

Trends R' Us. M.D. Paine. Paine, Ledge and Associates, North Vancouver, BC.

In environmental monitoring programs, trends (monotonic or progressive increases or decreases in variable values over time) are usually of interest after 5 years of data/sampling. Trends for monitoring variables (Y) are usually expressed as non-parametric correlations (Spearman's rank correlation {rs} or Kendall's tau {t}) between Y and time (t = X). The non-parametric correlations have limited power with $n < 10$ years for trends measured at a single location or time of year (season), but may have substantial power when trends are measured at multiple locations or seasons. I will review tests for comparing non-parametric trends among locations or seasons and other options for time series analyses. The review will include tests of time differences (= step changes) other than trends, and reversal of time and space for analysis of spatial gradients. I will provide handouts with methods for some generally applicable and widely used tests, although I will not discuss methods in detail. I will discuss some unconventional applications, and also some limitations (especially for software available on the web), for various tests or approaches. I would also like some feedback on the tests or approaches that other investigators use, since the choice of methods may not be obvious, and is often a matter of convenience, preference or (sometimes ill-advised) regulatory requirements or recommendations.

Evaluating short time series with a modified Mann-Kendall trend test. K.M. Somers[1] and M.F. Bowman[2]. [1]Ontario Ministry of the Environment, Environmental Monitoring and Reporting Branch,

Dorset, ON; and [2]Department of Zoology, University of Toronto, Toronto, ON.

Evaluating trends over time remains a challenge because of the relatively short length and non-linear nature of many time series. In the water-quality literature, the Mann-Kendall trend test is often used to assess monotonic trends in short data series. Although this test is simple to calculate and based on rank-order nonparametric statistics, it is rarely used with biological data. The test's lack of popularity may also reflect the somewhat restrictive, monotonic trend hypothesis that is evaluated. The Mann-Kendall trend test can be generalized using the Mantel test. This generalization provides opportunities to evaluate competing hypotheses of non-monotonic trends, step functions, and so on. In addition, the Mantel-test approach extends to tests of partial correlations and multivariate trend. The ability to test monotonic, non-monotonic, partial, and multivariate trend hypotheses should provide biologists with a valuable assessment tool. We demonstrate calculations for the traditional Mann-Kendall trend test and Mantel test variant using relatively short, time-series data for benthic invertebrates and water quality collected from Dorset-area lakes.

Gulfwatch blue mussel (*Mytilus edulis*) monitoring program: weight of evidence approach for spatial trend analysis. L.M White[1], P.G. Wells[2] and S.H. Jones[3]. [1]Ecosystem Research, Halifax, NS; [2]Environment Canada, Environmental Conservation Branch, Dartmouth, NS; and [3]Jackson Estuarine Laboratory, University of New Hampshire, Durham, NH.

Gulfwatch is an international blue mussel (*Mytilus edulis*) tissue contaminants monitoring program involving three states and two provinces. Gulfwatch has measured levels of PAHs, PCBs congeners, and DDT at 57 sites since 1991. Sampling occurs annually during the fall, but annual frequency (1 to 9 years) varies among sites. Spatial analyses of data from 1993 to 2001 examined trends in various ways: by jurisdiction (ranking), correlation with exact latitude (Kendall's tau b), and by watershed, and in geographic and latitudinal order (modified Mann-Kendall trend test). Trends were examined relative to annual sampling strategies. All significant trends showed that concentrations of organic contaminants decreased in a southern to northern direction in the Gulf of Maine. Spatial trends for PCBs and DDT were highly significant ($p < 0.0001$) for all variables and most sampling strategies. Spatial trends for PAHs were much weaker and significant only when all sites were analyzed ($p < 0.05$). The probability that concentration decreased with increasing latitude was around 0.50 for PCBs and DDT but ranged from 0.25 to 0.35 for PAHs. Weak spatial trends for PAHs likely reflect their chronic input from current and local sources as opposed to more historical and global inputs for PCBs and DDT. Future analyses will examine whether latitude is a proxy for population density as population density is much higher in the southern than northern Gulf of Maine. Further information on the Gulfwatch program can be found at http://www.gulfofmaine.org/gulfwatch/.

Biotic interactions in temporal trends (1992 to 2003) of organochlorine contaminants in Lake Laberge, Yukon Territory. M.J. Ryan[1], G.A. Stern[2], K.A. Kidd[3], M.V. Croft[2], S. Gewurtz[4], M. Diamond[4] and P. Roach[5]. [1]Stantec Consulting Ltd., Winnipeg, MB; [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; [3]Department of Biology, University of New Brunswick, St. John, NB; [4]Department of Geography, University of Toronto, Toronto, ON; and [5]Indian and Northern Affairs Canada, Yukon Region, Whitehorse, YT.

Recent declines in six organochlorine (OC) contaminant groups were measured in Lake Laberge, YT, following the closure of a commercial fishery. This study examined morphological, biochemical, population and OC contaminant data for fish and invertebrates across several temporal points between 1993 and 2003 to elucidate the causes for these OC declines. It was determined that growth dilution is a major factor influencing the decrease of OC. A broad decline in body lipid contents for most fish species has also contributed to the decline of contaminants although no such change was evident for

zooplankton. It is suspected that increases in populations of 7 fish species in Lake Laberge from 1991 to 1998 or climate changes over the 1990s may have contributed towards an increase in lake primary productivity as well as a shift in plankton community composition. Shifts in the zooplankton community have gone from an abundance of *Cyclops scutifer* in 1993 to dominance by *Diaptomus pribilofensis* in 2001. Biomagnification factors have increased for piscivorous fish yet remained stable for forage fish species over time. The overall food web magnification factors have increased for all 6 OC groups while the intercepts of these regression relationships are lower suggesting there is a less contaminated food base for the Lake Laberge food chain. Food web interactions have not changed over time with the exception of burbot (*Lota lota*) and possibly northern pike (*Esox lucius*). Only lake whitefish (*Coregonus clupeaformis*) exhibited a notable change in their food source. Considering the slow recovery of exploited lake trout (*Salvelinus namaycush*), fluctuations in populations, species characteristics and OC contaminant concentrations in the Lake Laberge ecosystem may continue for several years.

Assessment using data from local and regional reference sites over time: an example from Regional Aquatic Monitoring Program (RAMP). B.W. Kilgore[1], M. Davies[2], W.N. Gibbons[2] and P. McNamee[2]. [1]Stantec Consulting Ltd., Ottawa, ON; and [2]Hatfield Consultants Ltd., West Vancouver, BC.

The Regional Aquatic Monitoring Program (RAMP) was initiated in 1997 in response to mining development in the Athabasca oil sands region near Fort McMurray, Alberta. RAMP is an industry-funded, multi-stakeholder initiative that monitors the health of aquatic environments in the region. RAMP incorporates both stressor-based (e.g., water, sediment and fish tissue quality and water quantity) and effects-based (e.g., fish, benthos) monitoring approaches, and quantifies the quality of the receiving environments in tributaries to the Athabasca River. For each area to be developed, the program develops a minimum 3 year baseline of environmental quality for reaches about to be impacted, as well as for reaches upstream of the proposed development. Upstream reaches provide a site-specific control in a classic before-after-control-impact (BACI) context. Replication within reaches is typically quite high, particularly for surveys of benthos, and the multi-year BACI designs, thus, have exceptional power. RAMP also collects data in tributaries that will not be developed for many years. These "regional" reference areas provide an additional characterization of variability of the reference condition, and, through multivariate and clustering methods, are used to develop regional baseline descriptions of surface waters, sediments and benthos. These regional reference conditions are particularly valuable for water and sediment quality comparisons, where issues of replication and statistical power are problematic. Effects on the benthic community observed with the classic BACI designs are subtle when contrasted against the observed spatial variability.

Establishing a biological sediment assessment program for tracking long-term trends in the St. Clair River. T.S. Moran[1], J. Houtby[1], B.A. Zajdlik[2] and S. Munro[3]. [1]Pollutech Enviroquatics Ltd., Point Edward, ON; [2]B. Zajdlik and Associates, Rockwood, ON; and [3]Sarnia-Lambton Environmental Association, Sarnia, ON.

The St. Clair River was designated as an area of concern under the *Great Lakes Water Quality Agreement* due to a number of use impairments, including contaminated sediments. Ongoing monitoring has demonstrated improved control of point and non-point source discharges, and has led to an increased understanding of contaminant sources and their potential effects. Source control of contaminants has also reduced contaminant loadings to the aquatic sediments. In addition, completion of various remediation projects has removed contaminated sediments from localized areas in the St. Clair River. The Sarnia-Lambton Environmental Association, an environmental co-operative of local

industries, commissioned an integrated monitoring program to assess long-term trends of biological conditions in the St. Clair River and the potential for impacts associated with historical sediment contamination. The integrated study design incorporates sediment chemistry; sediment toxicity assessment through both *in situ* and laboratory toxicity testing; benthic macroinvertebrate community assemblage; and, the assessment of bioaccumulative potential targeting two species - mussel (*Dreissena spp.*) and round goby (*Neogobius melanostomus*). An overview of the study design and the results of the first iteration, or baseline study, to establish current ecological conditions, will be presented.

Modelling multiply censored data when more than 50% of observations are censored. B.A. Zajdlik[1], T. Fletcher[2] and J. Struger[3]. [1]B. Zajdlik and Associates, Rockwood, ON; [2]Ontario Ministry of the Environment, Ecological Standards Branch, Toronto, ON; and [3]Environment Canada, Environmental Conservation Branch, Burlington, ON.

Pesticide data were collected from the Don and Humber River watersheds during base flow periods and rainfall events from 1998 through 2002, inclusive. Observations were recorded as < detection limit (DL), detectable but not quantifiable or as the measured concentration and are therefore multiply censored. Measured concentrations frequently comprised < 50% of the observations for a given data set. Various hypotheses of the control-impact type were posited; many of these required control of nuisance variables such as the effect of year, etc. We describe a modelling approach to control these nuisance variables that makes optimal use of multiply censored data when more than 50% of observations are censored.

Guidance on statistics for determining endpoints in Canadian toxicity tests. J.B. Sprague[1] and R.P. Scroggins[2]. [1]Sprague Associates Ltd., Saltspring Island, BC; and [2]Environment Canada, Environmental Technology Centre, Ottawa, ON.

Environment Canada formed a Statistical Advisory Committee in 1993 to develop statistical guidelines for determining endpoints of toxicity tests. Efforts during a decade produced a guidance document on methods, as part of the Biological Test Method Series. Principal author is Dr. John B. Sprague, statistical advisors are Dr. Glenn F. Atkinson and Barry A. Zajdlik, and 42 other Canadian and International experts contributed to, reviewed, and commented on the various drafts. The basic objectives of the document are to provide: (i) guidance leading to a more standardized approach for calculating endpoints; (ii) information on the strengths and weaknesses of various statistical procedures; (iii) methods to assess whether experimental results provide definitive answers to the initial questions; (iv) examples of applying methods and interpreting results; and (v) guidance on recognizing and dealing with difficult data. The new document supplements the statistical methods and advice in the numerous toxicity test methods produced by Environment Canada, and gives specific advice for all the categories of tests. This includes single-concentration tests, quantal tests for lethality, point estimates for sublethal tests, dual-effect tests, and hypothesis testing. It advises on study design questions, statistical concepts, various common deficiencies, and certain kinds of problematic data. Fifteen technical appendices examine particular topics.

New statistical guidance from Environment Canada: changed approaches and software needs. J.B. Sprague[1] and R.P. Scroggins[2]. [1]Sprague Associates Ltd., Saltspring Island, BC; and [2]Environment Canada, Environmental Technology Centre, Ottawa, ON.

The biggest change is the emphasis on regression analysis for point estimates of sublethal effect (ICp). There are detailed instructions for linear and nonlinear regression. This replaces interpolation (the ICPIN procedure), and continues the movement away from hypothesis testing (NOEC/LOEC).

For quantal tests, recommended choices are: (i) logit/probit regression by maximum likelihood (ii) restricted Spearman-Kärber; and (iii) binomial method. Advice is offered on common deficiencies in current practice, such as narrow range of concentrations, abandoning geometric series during analysis, failure to check by hand plotting, confusion between "field" and "laboratory" replication, and procedures for warning charts. Explanations are given for some other topics: controls and reference toxicants; transformations; toxicity curves and median effective times. Advice is offered for "difficult" types of results: significant differences among endpoints; outliers; hormesis; and deviant patterns of effect. The following needs in software became apparent: (i) easy and obvious entry of data; (ii) default to logarithmic concentration; (iii) inclusion of the three quantal programs; (iv) a valid method for median effective time; (v) a tailored package for regression with choice of realistic models; (vi) Fisher's exact test and "Finney's tables", for single-concentration tests; and (vii) inclusion of a variety of conventional statistical tests including normality, homogeneity of variances, t-tests, analysis of variance, and a selection of multiple-range tests.

Making sense of fish estrogenicity data from refinery experiments. 2. Data analysis and interpretation. B.A. Zajdlik[1], J.P. Sherry[2], J.L. Parrott[2], S. Munro[3], T. Kierstead[4] and T.S. Moran[4]. [1]B. Zajdlik and Associates, Rockwood, ON; [2]Environment Canada, National Water Research Institute, Burlington, ON; [3]Sarnia-Lambton Environmental Association, Sarnia, ON; and [4]Pollutech Enviroquatics Ltd., Point Edward, ON.

We used a two-prong strategy to investigate the potential of wastewaters from several Ontario refineries to induce estrogenic responses in juvenile rainbow trout(*Oncorhynchus mykiss*). The potencies of end-of-pipe wastewaters were tested in 21 d bioassays using a static full-renewal design and caged trout were used to assess the recipient waters for estrogenicity and anti-estrogenicity in a pilot experiment. Plasma vitellogenin (Vg) was modelled as a function of refinery, injection status, gender, condition factor and liver somatic indices. Robust general linear models were required to deal with the unusual statistical distribution of plasma Vg generated by the *in situ* study. Mixed effects linear models were required to deal with the subsample information generated by the bioassay experiment.

Concentrations of metals in mussel samples collected at Canadian Coast Guard lightstations in coastal British Columbia. N. Healey and H. Damman. Department of Fisheries and Oceans, Institute of Ocean Sciences, Sidney, BC.

Mussels have been routinely collected since 2001 at 31 Canadian Coast Guard lightstations in coastal British Columbia to monitor potential marine inputs from contaminated soils and groundwater. Results to date will be discussed and compared to published results from similar studies as well as risk based criteria for the protection of human and ecological health. All results are reported in dry weight. Mean concentrations of Cu (8.7 ± 3.2 $mg \cdot kg^{-1}$) and Pb (8.7 ± 18.3 $mg \cdot kg^{-1}$) quantified in all near field samples (n = 56) are significantly greater than the mean concentrations of these contaminants in all far field samples (n = 22). The maximum near field results for Pb (93.6 $mg \cdot kg^{-1}$), Hg (2.84 $mg \cdot kg^{-1}$), Cu (27.7 $mg \cdot kg^{-1}$) and Sn (70.5 $mg \cdot kg^{-1}$) exceed the maximum concentrations of these metals in far field samples by a factor of 45, 10, 3 and 2 respectively. Near field concentrations of lead from 5 lightstations exceeded the 85th percentile concentration of lead reported by the NOAA National Status and Trends Program Mussel Watch.

Comparison of toxicity in field-collected sediment tests using different sediment to water ratios and equilibrium times using the freshwater amphipod *Hyalella azteca*. T.L. Watson-Leung[1] and M. Nowierski[2]. [1]Ontario Ministry of the Environment, Laboratory Services Branch Etobicoke, ON;

and [2]Ontario Ministry of the Environment, Standards Development Branch, Toronto, ON.

The Ontario Ministry of the Environment (MOE) compared the toxicity expressed in tests of field-collected sediment, using different sediment to water ratios and different equilibrium times. These tests were part of a study to evaluate the practical and technical merits of various standardized and validated testing methods. Three sediment to water ratios (S:WRs) were investigated: 1:1.75 vs. 1:4 vs. 1:67; and 1 d and 7 d sediment equilibration times were compared using the 1:4 S:WR only. Our investigation revealed that there was no significant difference in survival or growth of *Hyalella azteca* between the 1:1.75 and 1:4 S:WR exposures; however, the concentration of salts and metals was noticeably higher in the overlying water of the 1:1.75 S:WR vessels. Higher survival of amphipods was observed in the 1:67 S:WR exposure compared to the other two S:WR exposures and contaminant concentrations in the overlying water were the lowest and most stable of the three. No significant differences were observed in survival or growth between 1:4 S:WR tests after two different equilibrium periods in spite of the lack of equilibrium of some substances after 24 h. Results of these tests will be used to refine test procedures followed by the MOE and will be provided to Environment Canada for consideration of standard method amendments.

Lead sinker symposium/Symposium sur les plombs de pêche

Session chair/Président: Tony Scheuhammer

Environmental impacts of lead fishing sinkers and jigs in Canada. T.M. Scheuhammer. Environment Canada, Canadian Wildlife Service, Ottawa, ON.

The non-recoverable release of lead into the environment from lost fishing sinkers, jigs, and other lead-based terminal tackle, and their toxic impacts on common loons (*Gavia immer*) and other wildlife, has been an ongoing issue of concern in Canada since the early 1990s. In 2003, the Canadian Wildlife Service published a scientific review of this issue, titled "Lead fishing sinkers and jigs in Canada" that reviewed their use patterns and toxic impacts on wildlife. This review concluded that the accidental loss of lead sinkers and jigs by Canada's estimated 5 million recreational anglers represented about 500 tonnes of non-recoverable lead released into the environment every year; (i) that this release is a substantial percentage (14%) of the total estimated non-recoverable releases of lead in Canada; (ii) that, in places where studies have been done in Canada and the U.S., lead poisoning from sinker/jig ingestion typically accounts for 20 to 30% of total reported mortality of adult common loons on their breeding grounds in locations where recreational angling activities are high; (iii) that lead sinker poisoning is often the leading cause of death reported for adult common loons during the breeding season; (iv) that there is insufficient knowledge to conclude whether such lead poisoning may cause population-level impacts on loons; and (v) that numerous viable non-toxic sinker/jig products are available. This presentation will summarize the rationale behind these conclusions; and address some of the more common questions and criticisms that have been voiced regarding these conclusions.

The abundance of lost and discarded fishing tackle in the United States. A.E. Duerr[1], S. DeStephano[2] and C.T. Franson[3]. [1]Vermont Cooperative Fish and Wildlife Research Unit, University of Vermont, Burlington, VT; [2]Massachusetts Cooperative Fish and Wildlife Research Unit, University of Massachusetts, Amherst, MA; and [3]U.S. Geological Survey, National Wildlife Health Center, Madison, WI.

Lost and discarded fishing tackle poses a hazard to waterbirds and other wildlife. In the United Kingdom, lead poisoning due to ingestion of fishing sinkers in mute swans (*Cygnus olor*) led to a ban

From 1989 to 2005, nearly 1000 common loons (*Gavia immer*) found dead or moribund were examined as part of a New England wide mortality study. We have also participated in a regional study that involved banding and sampling over 1000 live, breeding loons. Results confirm that much summer loon mortality is due directly or indirectly to human activities on breeding lakes. Major findings included numerous deaths from trauma, fishing gear, and fungal respiratory disease. Although fish-hooks and lures appear to be minor causes of mortality, entanglement in monofilament line and heavy metal toxicosis (lead) from ingested sinkers and jigs accounted for much of the observed mortality. The magnitude of these problems; steps that can be taken to educate anglers; and the availability of suitable non-toxic alternatives will be discussed.

Mortality factors in common loons (*Gavia immer*) from maritime Canada. P-Y. Daoust. Department of Pathology and Microbiology, University of Prince Edward Island, Charlottetown, PE.

Between 1996 and 2005, we examined the carcasses of 83 common loons (*Gavia immer*) found dead in the Maritime provinces. Adults represented the majority of these carcasses (67 birds). Chronic or, less commonly, acute lead poisoning was diagnosed in 20 adults (30% of adult birds, 24% of total), representing the most common cause of mortality. The average concentration of lead in kidneys of these birds was 47.9 $mg \cdot kg^{-1}$ (wet weight) (range, 4.3 to 105 $mg \cdot kg^{-1}$). Fishing lure material was found in the gizzard of all 20 birds, including a distinct lead sinker or swivel in 16 of them. Trauma, acute or chronic, was the second most common cause of death (16 birds, 19% of total) and included aggressive inter- and intraspecific interactions, collisions with obstacles in flight, gun shots, and possible boat strikes. Oil contamination was identified in 11 birds (13% of total), the majority of which washed up on Sable Island, off the coast of Nova Scotia. These results are similar to those of a previous study done in this region and covering 1992 to 1995. Besides being the most common cause of mortality identified in common loons, lead poisoning also seems to be the most easily preventable.

Loon mortality in Michigan 1987 to 2004. T.M. Cooley. Michigan Department of Natural Resources, Wildlife Division, Lansing, MI.

Mortality factors of the common loon (*Gavia immer*) in Michigan were determined by the post-mortem examination of birds submitted by the public and State and Federal Agency personnel to the Michigan Department of Natural Resources Wildlife Disease Lab. Over the 18 year period, two hundred and four dead loons were examined and the leading causes of death were trauma (48), drowning (46), lead poisoning (45), and aspergillosis (21). There were many types of trauma observed, including deaths due to intraspecies competition, and collisions with roads, houses, and watercraft. Most of the traumatic cases examined at the Lab occurred on Inland Lakes. The primary cause of drowning was due to birds becoming entangled in fishnets. Most of the drowning cases examined at the Lab occurred on the Great Lakes. Lead poisoning was caused by jigs (26), sinkers (7), unidentifiable lead pieces (5), splitshot (3), no identifiable lead (3), and spent lead shot (1). Most of the drowning cases examined at the Lab occurred on the Great Lakes with the majority originating from Lake Michigan. Livers and kidneys were analyzed for metals from all birds that were examined. The majority of the mortality in the common loon in Michigan occurred in the adult age class.

Necropsy findings in adult common loons (*Gavia immer*) found dead in Ontario between April and November, 1989 to 2005. G.D. Campbell[1], I.K. Barker[1] and E.M. Addison[2]. [1]Canadian Cooperative Wildlife Health Centre, University of Guelph, Guelph, ON; and [2]Ontario Ministry of Natural Resources (retired), Maple, ON.

Common loons (*Gavia immer*) were examined at necropsy for body condition, cause of death,

significant disease conditions, parasite burdens and exposure to lead and mercury. Important causes of death included: trauma due to gunshot, drowning or collisions with boats, cars, roads or power lines (43/134), lead poisoning (33/134), ingestion of fish hooks, lures, fishing line or other foreign objects (13/134), infectious (bacterial, fungal, parasitic) diseases (20/134), emaciation (9/134) and miscellaneous other conditions (16/134). Tissue lead levels in lead-poisoned birds ranged from 17 to 270 $mg \cdot kg^{-1}$ dry weight in the liver and 9 to 480 $mg \cdot kg^{-1}$ (dw) in the kidney. In 29/33 lead-poisoned birds, either a lead fishing weight or some other fishing tackle was present in the gizzard. Mean weight of lead-poisoned birds was 3.36 kg. Body condition was judged to be emaciated in 17/32 birds, fair-poor in 14/32 and good in 1/32. During the period 1998 to 2004, an estimated 12,000 common loons died on Lake Huron and Lake Erie during the autumn months as a result of intoxication with Type E botulinum toxin. None of the birds necropsied in Ontario during these episodes had fishing weights in their gizzards and no lead was detected in tissue in a sample of these birds.

Use of population endpoints in environmental risk assessment. W.N. Beyer. U.S. Geological Survey, Patuxent Wildlife Research Center, Laurel, MD.

Risk assessors sometimes differ over the use of population endpoints in environmental risk assessments: assessors who think in terms of long-term survival of particular species tend to support a population approach whereas environmental regulators tend to think in terms of habitat quality. Much of the controversy associated with the issue comes from the different meanings associated with the word population. The word may used variously to mean a local group of organisms, a larger group, or to all members of the species. This presentation reviews regulations and guidance documents on risk assessment from the U.S. Environmental Protection Agency (EPA) that are relevant to the issue. Although previous guidance documents from the EPA seemed to encourage a shift in emphasis toward greater reliance on population assessments, recent guidance seems to support the continued use of more traditional endpoints.

Hazards to humans associated with the making and use of lead items. E. Nieboer. Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON.

Because of the relatively low melting point of lead (328 °C), it is easy to generate toxic fumes of this metal. Exposures to lead-containing aerosols associated with the pouring of molten lead and the working of lead objects (i.e., grinding, smoothing) have been assessed and reported in the scientific literature. Chemical characterization of the surface of weathered lead bullets provides proof that under oxidizing conditions in the presence of oxygen, carbon dioxide and moisture, lead ions, lead oxide and lead complexes of carbonate and the hydroxide ion are formed. Leaching studies by us and others have shown that the lead from objects such as lead pellets and dental film is bioaccessible (i.e., available for uptake). Case study reports will be used to illustrate the toxic consequences of inhaling lead fumes and particulate aerosols of lead and the personal protection equipment needed. Similarly, a review of case studies describing the consequences of ingesting lead pellets, bullets or sinkers is summarized. Whole-blood lead concentrations observed in such instances will be compared to the level at which medical concern and review begin, namely 0.48 $\mu mol \cdot L^{-1}$ (100 $\mu g \cdot L^{-1}$).

Best student paper awards/Prix pour les meilleurs exposés par les étudiants

Best Platform Paper Award

Marie-Line Gentes, Department of Veterinary Pathology, University of Saskatchewan, Saskatoon, SK.

Blowfly infestation of tree swallow (*Tachycineta bicolor*) nests on the Athabasca oil sands.

Best Poster Paper Award

Natacha Hogan, Department of Biology, University of Ottawa, Ottawa, ON.

Assessing thyroid-disrupting effects of estrogenic compounds on amphibian metamorphosis.

Other Platform Paper Awards

Second: Marilynn Kullman, Department of Biology, University of New Brunswick Saint John, NB.

Third: Rebekah Rooney, Department of Entomology, University of Manitoba, Winnipeg, MB.

Honourable Mention: Colin Khan, Department of Biology, Queen's University, Kingston, ON; and Lisa Kraemer, Institut National de la Recherche Scientifique – Eau, Terre et Environnement, Université du Québec, Sainte-Foy, Qc.

Other Poster Paper Awards

Second: Laura Hornung, Department of Biology, Canisius College, Buffalo, NY.

Third: Marcel Pinheiro, Department of Biology, University of Waterloo, Waterloo, ON.

Honourable Mention: Leo Lin, Toxicology Centre, University of Saskatchewan, Saskatoon, SK; and Sarah Van Es, Department of Biology, Wilfrid Laurier University, Waterloo, ON

Acknowledgements

There were 46 papers that were presented for the Best Student Paper Awards. We thank Paula Dehn who chaired this competition, and the following judges: Farida Bishay, Les Burridge, Gordon Craig, Monique Dubé, Jennifer Froese, Guy Gilron, Kats Haya, Kathleen Hedley, Karen Kidd, Deborah MacLatchy, Tim Moran, Cheryl Podemski, Sylvie St. Jean, Paula Siwik and Judit Smits.

Short courses/Cours de formation

Six courses were held during the workshop, these were:

The art of presenting and discussing science

Instructors: Debra LacLatchy and Karen Kidd, Department of Biology, University of New Brunswick, St. John, NB; and Joanne Parrott, Environment Canada, National Water Research Institute, Burlington, ON.

Aquatic toxicology *in vitro*: methodologies and applications

Instructors: Lucy Lee, Department of Biology, Wilfrid Laurier University, Waterloo, ON; Kristin Schirmer, Department of Cell Toxicology, UFZ-Centre for Environmental Research, Leipzig, Germany; and Vivian Dayeh, Department of Biology, University of Waterloo, Waterloo, ON.

Introduction to soil toxicology

Instructors: Natalie Feisthauer and Jill Crumb, Stantec Consulting Ltd., Guelph, ON.

Introduction to the analysis of variance table

Instructor: Barry Zajlik, Zajdlik & Associates, Rockwood, ON.

The science of risk communications: building trust and credibility in difficult times

Instructors: Trevor Smith Diggins, Frontline Corporate Communications Inc., Kitchner, ON; and Ronald Brecher, Globaltox International Consultants, Inc., Guelph, ON.

Canadian water quality guidelines for the protection of aquatic life: how these guidelines are derived and how to apply and interpret them

Instructors: Susan Roe and Uwe Schneider, Environment Canada, National Guidelines and Standards Office, Gatineau, QC.

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Bandelj, Emil
Bard, Shannon
Bardai, Ghalib
Bartlett, Adrienne
Bayer, Barb
Bennett, Pamela
Bennie, Don
Benoit, Nadine
Beyer, Nelson
Bird, Glen
Birkholz, Deib
Bishay, Farida
Bishop, Jim
Blazer, Vicki
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Bols, Niels
Borgmann, Anne
Borgmann, Uwe
Bowman, Michelle
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Brereton, Christine
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Brotman, Mark
Brown, Adriana
Brown, Scott
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Bruno, Joy
Brunton, Alex
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Buchkowski, Joy
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Burridge, Les
Cairns, Meghan
Campbell, G. Douglas
Campbell, Linda
Canaria, Edmund
Casey, Richard
Caux, Pierre-Yves
Chalmers, Ben
Charlton, Murray
Chong-kit, Richard
Ciborowski, Jan
Clarke, Lauren
Coker, George
Colavecchia, Maria
Cole, Gord
Collins, Art
Constable, Miles
Cooley, Thomas
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Craig, Gordon
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Delong, Eric
Demers, Marc
Diep, Ngan
Dixon, Brian
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Doe, Ken
Donald, David
Donaldson, Garry
Drake, Manon
Driedger, Kimberlea
Drouillard, Ken
Dubé, Monique
Duerr, Adam
Dunn, Allison
Dupuis, Hélène
Dussault, Éve
Dutton, Mike
Elliott, Garth
Elphick, James
Elshayeb, Monalisa
Eyding, Nicola
Fabbri, Elena
Fahraeus Van Ree, Goverdina
Farwell, Andrea
Feisthauer, Natalie
Ferguson, Clay
Ferguson, Ken
Feron, Jenny-Marie
Fletcher, Tim
Fletcher, Rachael
Flood, Ken
Florek, Charlene
Fowlie, Adrienne
Fox, Don
Fraikin, Chris
Frank, Richard
Franklin, Natasha
Franson, Chris
Froese, Jennifer
Galehdar, Zahra
Gec, Bob
Gentes, Marie-line
George, Tara
Georgiades, Eugene
Gibbons, Wade
Gilbertson, Mary-kate
Gilbertson, Michael

Gillis, Patricia
Gilron, Guy
Glozier, Nancy
Goddard, Chris
Goldacker, Suzanne
Golding, Lisa
Goold, Andrea
Grapentine, Lee
Gravel, Amelie
Gray, Michelle
Green, Joan
Green, Warren
Gurska, Yola
Haffner, Douglas
Hagen, Mike
Hale, Beverley
Hall, Chris
Hall,Ronald
Hamilton, Hugh
Hamoutene, Dounia
Harttrup, Nancy
Harwood, Manon
Hatton, Elizabeth
Havel, Christine
Haya, Kats
Healey, Norm
Hedley, Kathleen
Hill, Jonathan
Hodson, Peter
Hogan, Natacha
Holdway, Douglas
Holzapfel, Angela
Honkanen, Jani
Hornung, Laura
Houtby, Jeff
Hull, Ruth
Hunter, Kim
Huras, Craig
Hyatt, Wayne
Jaagumagi, Rein
Jackman, Paula
Jackson, Francis
Jamieson, Lee
Janz, David
Ji, Bo
Jiapizian, Paul
Johnston, Blair
Joncas, Johane
Jun, Henry
Kaminski, Edward
Karaga, Rick
Kavanagh, Richard
Keeling, Suzanne
Keene, Joel
Khan, Colin
Kidd, Karen
Kierstead, Ted
Kilgour, Bruce
Kim, Kay
Kirk, Jennifer
Klinck, Joel
Knight, Brendan
Kohli, Mohan
Kraemer, Lisa
Kroetsch, Jim
Kruper, Nancy
Kuley, Ed
Kullman, Marilynn
Kussatz, Carola
Kwiatkowski, Derrick
Lampi, Mark
Lee, Lucy
Lee, Peter
Levesque, Lucie
Lewin, Andrew
Lin, Leo
Lopez, Mirey
Lowell, Richard
Mackinnon, Mike
MacLatchy, Deborah
Maddison, Linda
Maher, Sue Ellen
Maloney, Shirley
Manion, Nathan
Marlatt, Vicki
Martel, Louis
Martyniuk, Chris
Marwood, Christopher
Matteau, Isabelle
Mayer, Tanya
McCann-Cermak, Janet
McCarty, Lynn
Mccubbin, Neil
McGeer, Jim
Mckee, Paul
McKenna, Gerry
McKnight, Elaine
McMaster, Mark
Middelraad, Irene
Middlebrook, Ken
Milani, Danielle
Miller, Jennifer
Milne, Jacqui
Moccia, Richard
Mok, Sandra
Moody, Mary
Moon, Thomas
Moore, Dan
Moran, Tim
Morgan, Tammie
Morita, Carrie
Moseley, Scott
Muir, Derek
Munro, Scott
Munson, Barry
Muscatello, Jorgelina
Muter, Mary
Muttray, Annette
Nagpal, Narender
Nairn, Rob
Naylor, Steve
Nero, Vincent
Ng, Robert
Nieboer, Evert
Niimi, Arthur
Norwood, Warren
Nowierski, Monica
Ogletree, Garry
Okonski, Alexander
Olaveson, Kelly
Orr, Patti
Ostapyk, Kim
Paine, Michael
Parks, Derek
Parks, John
Pastershank, Georgine
Peterson, Amy
Petrie, Scott
Phillips, Robert
Pinheiro, Marcel
Pintwala, Claudette

Plant, Wes
Podemski, Cheryl
Poirier, David
Pokras, Mark
Portt, Cam
Potter, Kelly
Power, Mary
Princz, Juliska
Purdy, John
Raeside, Ashley
Rahn, Jessica
Reid, Gregor
Reid, Jim
Rickwood, Carrie
Ring, Bonna
Robertson, Erin
Rooney, Rebekah
Rosaasen, Arden
Roshon, Roxana
Russo, Nick
Rutherford, Les
Rutley, Neil
Ryan, Michael
Sabo, Elisabeth
Salisbury, Carol
Samoiloff, Clifton
Santavy, Sonya
Scheuhammer, Tony
Schirmer, Kristin
Schnabel, Sabine
Schneider, Uwe
Schroeder, Julie
Schuster, Roseanne
Schwartz, Melissa
Scott, Jason
Scroggins, Rick
Sein, Rod
Servos, Mark
Sharpe, Rainie
Shealy, Laura
Shelast, Bob
Sherry, Jim
Shin, Mari
Shipton, Jeff
Siwik, Eden
Siwik, Paula
Smith, Alasdair
Smith, Cindy
Smith, Daniel
Smith, Scott
Smith, Stephen
Smits, Judit
Solomon, Keith
Somers, Keith
Spafford, David
Spafford, Mark
Sprague, John B.
Spry, Doug
St. Jean, Sylvie
Starodub, Mary Ellen
Stephenson, Gladys
Stoughton, Sarah
Svoboda, Lewina
Swisterski, Anna
Szalinska, Ewa
Tang, Armando
Taylor, Darrell
Taylor, Jacqueline
Taylor, Ken
Taylor, Lisa
Teather, Kevin
Tetreault, Gerald
Thomas, Vernon
Tremblay, Ashley
Trudeau, Vance
Trudel, Lise
Truelson, Robert
Turcotte, Dominique
Tytka, Nick
Vallieres, Genevieve
Van den Heuvel, Mike
Van der Vliet, Leana
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Van Geest, Jordana
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The Proceedings of each Annual Aquatic Toxicity Workshop have been published in a series of Technical Reports listed below. These Proceedings are generally provided to each Workshop participant, and are also sent to selected libraries, government departments and other agencies. Copies of 4th and subsequent Proceedings may be available for a charge, as photocopies or fiche, from Micromedia Limited, 240 Catherine Street, Suite 305, Ottawa, ON, K2P 2G8 (613-237-4250).

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