Proceedings of the 28th Annual Aquatic Toxicity Workshop: September 30 - October 3, 2001, Winnipeg, Manitoba

Comptes rendus du 28e atelier annuel sur la toxicité aquatique du 30 septembre au 3 octobre 2001, Winnipeg, Manitoba

Editors/Éditeurs

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2001

Canadian Technical Report of Fisheries and Aquatic Sciences 2379

Rapport technical canadien des sciences halieutiques et aquatiques 2379

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Canadian Technical Report of Fisheries and Aquatic Sciences

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PREFACE/PREFACE

The 27th Annual Aquatic Toxicity Workshop was held at the Delta Winnipeg Hotel in Winnipeg, Manitoba, September 30 to October 3, 2001. The Workshop included 1 plenary presentation, 108 platform and 43 poster papers. Total attendance was 250. The banquet speaker was Elizabeth May, Executive Director of the Sierra Club of Canada.

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 run by an incorporated National Steering Committee, and the Proceedings are published with the support of the Department of Fisheries and Oceans.

Le 28e atelier annuel sur la toxicité a eu lieu L'Hôtel Delta Winnipeg, Winnipeg, Manitoba, 30 septembre au 3 octobre 2001. Le atelier a donné lieu a 1 communication lors de séances plénières, 108 exposés d'invités d'honneur 43 communications par affichage. 250 personnes ont assisté au atelier. La conférencière de la soirée banquet était Élizabeth May, Directrice Exécutive du Sierra Club of Canada.

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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PLENARY SESSION/SÉANCE PLÉNIÈRE

A. Schafer. Director, Centre for Professional and Applied Ethics, University of Manitoba, Winnipeg, MB

The Social Responsibilities of the Scientist

Do scientists is general and aquatic toxicologists in particular have special responsibilities to society? If so, why? And just what are those responsibilities? When an aquatic toxicologist is employed by a corporation or by a government to do research, and when that research uncovers dangers to the public or to the environment, does he or she have an obligation to ensure that this information becomes available to all whose lives or health may be affected? Even if this means "blowing the whistle" on one's employer/client? Which environmental risks are worth taking for which benefits, and who should decide" How can we escape from "the tragedy of the commons"? This presentation will develop a set of interconnected themes relating to environmental ethics from the perspective of aquatic toxicology.

PLATFORM PRESENTATIONS/SÉANCE EXPOSÉS

Pulp and Paper Environmental Effects Monitoring

Session Co-chairs: K. Hedley and C. Portt

A National Assessment of Aquatic Effects of Pulp and Paper Mill Effluent Discharges. I.K. Ellis[1] and R.B. Lowell[2]. [1]Environment Canada, National EEM Office, Hull, QC; [2]Environment Canada, National EEM Office and National Water Research Institute, Saskatoon, SK.

The Canadian pulp and paper industry has recently completed their second round (Cycle 2) of Environmental Effects Monitoring (EEM) studies, as required under the *Pulp and Paper Effluent Regulations* of the federal *Fisheries Act*. Environment Canada will complete a national assessment of the effects of pulp and paper mill effluent in aquatic environments and has begun a preliminary analysis of this data. The goal of the data analysis is to understand the potential types and magnitude of effects that may occur and to recommend any improvements to the monitoring approaches used in the program. Some examples of this analysis include statistical comparisons of "effects" endpoints (e.g., taxa richness) between reference and exposure sites to determine the presence, spatial extent and magnitude of effects and the influence of mill process on the type of "effect" found. Environment Canada will review the literature and compare historical data on the types of effects generally associated with pulp mills to Cycle 2 results to examine any similarities in typical response patterns in fish and benthic invertebrate communities and to determine whether or not the situation is improving. The focus of this presentation is to provide an overview of the results from the preliminary analyses of Cycle 2 data and to provide an historical review of pulp and paper mill effects in aquatic environments.

National Assessment of the Effects of Pulp and Paper Mills on Benthic Invertebrate Communities: Meta-analyses of the National EEM Database. R.B. Lowell[1], L.C. Grapentine[2], J.C. Culp[3] and T.B. Reynoldson[2]. [1]Environment Canada, National EEM Office and National Water

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

Canada's National Environmental Effects Monitoring (EEM) Program has recently completed the second cycle of pulp and paper mill monitoring. Environment Canada has been analyzing these results to help interpret measured effects on benthic invertebrates and fish in freshwater and marine receiving waters. Meta-analytical techniques are being applied to determine the magnitude of effects that have been measured and multivariate techniques are being used to determine patterns in effects and correlations among the different core endpoints. These approaches, conducted at a national level, allow questions to be addressed that cannot be evaluated at the level of the individual mill. This talk will focus on the meta-analyses of the benthic invertebrate community results. The key response variables are the magnitudes of measured effects on core invertebrate endpoints (e.g., abundance, taxa richness, distribution of individuals among taxa). Potential responses to effluents will be evaluated on a national scale by making statistical comparisons among different mill groupings. Some of the issues that will be addressed include the influence of mill process type, effluent treatment type, habitat, and study design on the magnitude of measured effects. The question of what might be considered large versus small effects will also be addressed.

Responses of Benthic Invertebrate Communities to Pulp and Paper Mill Effluents: Multivariate Analyses of Pooled Data from the National EEM Program. L.C. Grapentine[1], R.B. Lowell[2], T.B. Reynoldson[1] and J.C. Culp[3]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Environment Canada, National EEM Office and National Water Research Institute, Saskatoon, SK; [3]Environment Canada, National Water Research Institute, Saskatoon, SK.

Under the national Environmental Effects Monitoring program for the pulp and paper industry, benthic invertebrate studies conducted between 1997 and 2000 and aimed at determining the degree and spatial extent of effects due to effluents have been reported for approximately 100 mills across Canada. From these studies, data on the relative abundances of macroinvertebrate families together with matching mill and environmental descriptors were accessible and validated for the majority of mills, with over 900 sampling locations. These data were combined into a single super matrix to allow a more powerful test (than single mill analyses) for effects of mill effluents on benthic communities, and to assess whether mill characteristics (pulping process, effluent treatment) and selected environmental factors influence the responses of benthic communities to effluent exposure. Analyses were by a series of multivariate ordination and cluster analyses performed on the matrix partitioned into various mill groups. Results will be presented that [1] determine if benthic communities exposed to near field levels of mill effluent show a consistent difference in their structure compared to unexposed communities, [2] compare the size of this effect with the natural variability in community structure, and [3] identify important mill and habitat factors related to the effect.

National Science Assessment of EEM Pulp and Paper Mill Effects - the Fish Survey. K.R. Munkittrick[1], S. Ribey[2], R.B. Lowell[2,3] and M.E. McMaster[4]. [1]Department of Biology, University of New Brunswick, St. John, NB; [2]Environment Canada, National EEM Office, Hull, QC; [3]Environment Canada, National Water Research Institute, Saskatoon, SK; [4]Environment Canada, National Water Research Institute, Burlington, ON.

Cycle 2 of the pulp and paper EEM program was completed last year and since that time, Environment Canada has completed a preliminary evaluation of the results that were reported in the mills interpretative reports. The four main response patterns observed were responses that demonstrated that fish exhibited [1] an increase in condition, liver size and gonad size, [2] an increase in condition and/or liver size, but a decrease in gonad size, [3] a decrease in condition, liver size and gonad size, and [4] a mixed or inconsistent response. Response [1] is indicative of nutrient enrichment, [2] is consistent with metabolic disruption, and [3] is consistent with food limitation (or food enrichment at the reference site). Subsequent to these preliminary analyses, a national science assessment of pulp and paper mill effects was initiated, which involved data extraction in order to conduct meta- and multivariate analyses on a national scale. The primary objective of the analyses for the fish survey is to examine the magnitude of the effects on the key fish endpoints (growth, reproduction, condition and survival) as influenced by mill process and effluent treatment type, as well as by certain fish characteristics (e.g., sex, species). The analyses will also include an evaluation of what might be considered large versus small effects in terms of the level of responses across Canada. This presentation will provide an overview of the results to date of the science assessment for the Cycle 2 fish survey.

Pulp and Paper Environmental Effects Monitoring (EEM) in British Columbia: Cycle 2 Review, 1997-2000. E. Dobson, W.N. Gibbons and L.W. Dwernychuk. Hatfield Consultants Ltd., West Vancouver, BC.

Twenty-three pulp and paper mills in British Columbia discharge into aquatic receiving environments and are, therefore, required to perform EEM programs. Prince George Pulp and Paper Ltd. and Intercontinental Pulp Co. (PG/IC), owned by Canadian Forest Products Ltd., combine effluent treatment and discharge through one outfall. Therefore, EEM programs were conducted at 22 discharges in British Columbia for Cycle 2, 1997 to 2000 (combined programs were conducted for mills in close proximity to each other, e.g., upper Fraser River mills). Some mill studies revealed an inhibition of invertebrate communities, a few mills exhibited enhanced fish condition and/or invertebrate communities. Mills exhibiting lower sublethal toxicity endpoints did not necessarily correlate with findings in receiving environments. Dioxin and furan monitoring programs measure historical contamination, concentrations of these compounds do not necessarily relate to effects observed in biological communities. There is evidence of improvement in near-field areas of mills that exhibited inhibition during historical surveys. These improvements are most likely the result of mill upgrades, secondary treatment of effluent (marine mills), and the substitution of elemental chlorine with chlorine dioxide.

Pulp and Paper Environmental Effects Monitoring (EEM) in the Prairie and Northern Region: A Synopsis of Cycle 2 Results (1996 to 2000). S.A. Blenkinsopp[1], J. Ferone[1] and M. Miyagawa[2]. [1]Environment Canada, Toxic Substances Division, Edmonton, AB; [2]Environment Canada, Pollution Prevention and Assessment Division, Vancouver, BC.

The national Environmental Effects Monitoring (EEM) program falls under the *Pulp and Paper Effluent Regulations* of the *Fisheries Act*. The program requires that the ten mills in the Prairie and Northern Region (PNR) which discharge to the aquatic environment conduct regular EEM studies on their receiving environments in order to assess and monitor effects potentially caused by the mills' effluent. Five mill process types are found in PNR: bleached kraft, unbleached kraft, bleached chemi-thermomechanical pulp (BCTMP), integrated thermomechanical pulp/de-inked pulp, and groundwood/sulphite. In Cycle 2 (1996 to 2000), nine mills were required to do a fish field survey, and all ten were required to do a benthic invertebrate field survey. Samples for the field surveys were collected in upstream reference area(s), and below the mill discharge in downstream exposure area(s). In addition, the mills were required to submit semi-annual sublethal toxicity tests on mill effluent using *Pimephales promelas*, *Ceriodaphnia dubia*, and *Selenastrum capricornutum*. The Cycle 2 fish survey, benthic invertebrate survey and sublethal toxicity results are summarized and discussed.

Ontario Region Case Studies of Sublethal Toxicity Testing as an Environmental Effects Monitoring Tool. A.I. Borgmann[1], R.P. Scroggins[2], J.A. Miller[3] and J.B. Sprague[4]. [1]Environment Canada, Environmental Protection Branch, Downsview, ON; [2]Environment Canada, Environmental Technology Centre, Ottawa, ON; [3]Miller Environmental Sciences Inc., Innisfil, ON; [4]Sprague Associates Ltd., Salt Spring Island, BC.

Data collected through Environment Canada's Environmental Effects Monitoring Program in three Ontario case studies, showed that sublethal toxicity tests gave reasonable predictions of relative effects of effluent in the receiving water. In the first situation at a pulp mill, the zone of potential effects estimated from toxicity data agreed with the results of a benthic field survey. In the second case, discharges from two pulp mills and a power generation station near Thunder Bay were shown to have overlapping zones of potential effect in Cycle 1, based upon tests of *Ceriodaphnia* reproduction. The overlap disappeared in Cycle 2, after installation of secondary treatment at the mills. The final case study involved four pulp mills discharging into Twelve Mile Creek, in the Niagara region. Toxicity tests indicated that one mill contributed up to 94% of the toxic loading, although its volume of effluent was only 22% of the total. Results of these studies emphasize the usefulness of sublethal toxicity testing as a tool in the EEM program.

Introducing a Scheme for Comparing Sublethal Toxicity and Field Survey Results from EEM Studies. A.I. Borgmann[1], M. Moody[2] and R.P. Scroggins[3]. [1]Environment Canada, Environmental Protection Branch, Downsview, ON; [2]Saskatchewan Research Council, Saskatoon, SK; [3]Environment Canada, Environmental Technology Centre, Ottawa, ON.

A scheme for the evaluation of the possible relationship between the laboratory sublethal toxicity tests and the field survey endpoints of EEM has been devised. Sublethal toxicity tests are assigned an effect score (1 to 5) based on the lowest IC_{25} for the secondary-treated effluent. For example, a score of 1 would imply no effect (the lowest IC_{25} is greater than or equal to 100% v/v effluent) while a score of 5 would imply a severe effect (the lowest IC_{25} is less than 1% v/v effluent). Field surveys of fish and benthos are assigned a score based on the number of significant mill-related effects in four relevant categories. The categories for benthos are abundance, number of taxa, abundance of enrichment indicator organisms and abundance of toxicity indicator organisms. For fish the categories are age, growth, reproduction and energy storage. In the field surveys, therefore, a score of 1 would imply no significant effects whereas 5 (severe) would imply significant differences in all four categories. Examples of a lake and a river study will be discussed to illustrate the effectiveness of this scheme. The majority of the 16

Ontario mills studied showed a strong lab-to-field correlation, plus or minus 1 point between the scores, following this assessment scheme.

Confirming Exposure of Seasonally Resident White Sucker (*Catostomus commersoni*) to Bleach Kraft Pulp Mill Effluent in a Northern Canadian River System. A.L. Langhorne[2], R.P. Schryer[1], S.M. Swanson[3], B.K. Firth[4], T. Kaptein[5] and K.T. Himbeault[1]. [1]Golder Associates Ltd., Saskatoon, SK; [2]Golder Associates Ltd., Yellowknife, NT; [3]Golder Associates Ltd., Calgary, AB; [4]Weyerhaeuser, Tacoma, WA; [5]Weyerhaeuser, Prince Albert, SK.

Weyerhaeuser undertook a white sucker (*Catostomus commersoni*) mark-recapture study on the North Saskatchewan River in 1998 as part of a Cycle 2 Environmental Effects Monitoring Program. The purpose was to address questions of white sucker exposure and residency within the effluent plume discharged by the Weyerhaeuser bleach kraft pulp and paper facility in Prince Albert, SK. In concert with the study, bile was collected from fish for resin acid analysis. The objectives were to: [1] assess the seasonal residency of white sucker in areas exposed to effluent during late summer and early fall, and [2] confirm exposure through the analysis of bile for resin acids.

The recapture rate for white sucker was 9.0%. Of these, the majority of the fish were both tagged and recaptured in the effluent plume, primarily in close proximity to their original capture location. Bile from white sucker in the exposure area also had detectable levels of five resin acids found in the mill effluent. Bile from fish captured in the reference area had detectable concentrations of two resin acids, but concentrations were low in comparison to exposure area fish.

Assessment of Stable Isotopes as Tracers of Fish Exposure to Pulp Mill Effluent. M.G. Dubé[1], L. Wassenaar[1] and S.A. Blenkinsopp[2]. [1]Environment Canada, National Water Research Institute, Saskatoon, SK; [2]Environment Canada, Environmental Protection, Edmonton, AB.

Stable isotopes of carbon ($d^{13}C$), nitrogen ($d^{15}N$), and sulfur ($d^{34}S$) have been used with increasing frequency to describe food web relationships amongst organisms and food sources. Stable isotope analyses also show promise as a technique to quantify exposure of aquatic biota to pulp mill effluents. Some pulp mill effluents have been shown to have isotopic signatures that are distinct from reference areas. Organisms that ingest effluent constituents can assimilate, along with their food sources, the isotopic signals of the contaminant into their tissues. In the first two cycles of the Canadian EEM Program, field surveys were conducted to assess the effects of pulp mill effluents on wild fish. In many cases the field surveys were confounded because of a lack of knowledge of fish mobility between reference and exposure areas. There is some evidence that stable isotopes of C, N, S, and chloride can be used as tracers in fish tissues to measure exposure to pulp mill effluent. Preliminary research is being conducted to assess the feasibility of using $d^{13}C$, $d^{15}N$, $d^{34}S$, and $d^{37}Cl$ as tracers of fish exposure to pulp mill effluent. Chloride has not been used before in this capacity.

The project consists of two components: [1] characterization of the isotopic signatures of Canadian pulp mill effluents, and [2] exposure of fish in the laboratory to assess tissue assimilation of stable isotopes. The first component of the project will address the question; do final effluents from different pulp mills have distinct isotopic signatures? The second component of the project will address the questions; do stable isotope signatures in fish tissues differ in fish exposed to 10% pulp mill effluent compared to control fish? Is this difference dependent upon exposure of the food source? (i.e., can effluent exposure result in differences in chloride signatures through water-borne exposure only). In the lab, hatchery-reared juvenile rainbow trout have been raised under control conditions and 10% treated pulp mill effluent for 8 weeks. Groups of fish within each treatment have been fed chironomids cultured under either control conditions or under effluent exposed conditions (10% PME). At the end of the exposure period, muscle and liver samples will be collected and analyzed for stable isotopes. Study design, preliminary results and conclusions will be presented and discussed.

Pseudoreplication and False Positives in Dredge Disposal and Other Environmental Assessments. M.D. Paine. Paine Ledge and Associates (PLA), North Vancouver, BC.

In assessments of areas to be dredged, *n* field samples are typically collected, composited, then split in the laboratory for chemical analyses or toxicity tests. The variance among lab reps is then used as the error variance for comparing the test site mean to a fixed standard (e.g., an environmental quality criterion) or to a mean from a reference or disposal area. This design or approach is pseudoreplication, and will lead to unacceptably high risks of false positives (i.e., concluding that there is a difference when there is not) whenever field variance is greater than lab variance. That conclusion will be demonstrated using simulations based on real lab and field variances. Analogous problems occur in other types of assessments. For example, fish catches from several sample reaches, gill net sets or trawls within an area may be pooled, with variance among individual fish used as the error variance for statistical tests. If there is added variance among reaches, sets or trawls, the risk of false positives will again be greater than the conventional 5% used for statistical testing. However, multiple reaches, sets or trawls may have to be pooled to provide adequate sample numbers or volumes. Some practical guidance will be provided based on the author's experience; that guidance will obviously be improved if others contribute their experience and data.

Toxicity Testing Methods - Development and Applications

Session Co-chairs: R.P. Scroggins and J. Schroeder

Metal Bioavailability as Determined by Diffusive Gradients in Thin Films (DGT) and Anodic Stripping Voltametry (ASV). J.J. McNee[1], A.J. Martin[1] and J. Crusius[1,2]. [1]Lorax Environmental Services Ltd., Vancouver, BC; [2]Department of Earth and Ocean Sciences, University of British Columbia, Vancouver, BC.

Metal concentrations in receiving waters are a significant concern in many environments worldwide. It is widely recognized that the concentration of "free" metal ion provides a better measure of metal toxicity than either total or dissolved concentrations. For example, in the presence of complexing ligands (such as naturally occurring organic compounds, EDTA, etc.), free metal concentrations are often substantially lower than their respective total or dissolved concentrations. Nonetheless, metal laden discharges are regulated on the basis of total metal concentrations. In this work, the bioavailability of a variety of metals was assessed in the presence of several complexing ligands by several different methods, including 48 h *Daphnia magna* bioassays, DGT (diffusive gradients in thin films) and ASV (anodic stripping voltammetry).

Results indicate that both DGT and ASV yield concentrations of "bioavailable" metals that correlate much better with toxicity to *D. magna* than do the total concentrations. These results suggest that regulation of metal concentrations in effluents and receiving waters in terms of "bioavailable" concentrations should be preferable to the current practice of regulation based on total metal content.

Creating a Model for Predicting Metal Toxicity to Invertebrates. J. Schroeder[1,2], U. Borgmann[3] and D.G. Dixon[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Ontario Ministry of the Environment, Etobicoke, ON; [3]Environment Canada, National Water Research Institute, Burlington, ON.

As part of the national MITE-RN (Metals in the Environment Research Network) program, our study attempts to predict toxicity of metals to *Hyalella azteca* by taking a similar approach to that used in the development of the biotic ligand model for fish. Short-term experiments were conducted to estimate binding constants for Ni and major ions to the freshwater amphipod, *H. azteca*. Each exposure was carried out under identical conditions in defined media and Ni accumulation in whole tissue was measured by graphite furnace atomic adsorption. The total amount of tissue (biotic ligand) available for binding was estimated by relating Ni in tissue to the metal concentration in solution. To estimate binding constants, *H. azteca* were exposed to a constant concentration of Ni in media of differing ionic composition. In each exposure, a single anion or cation was manipulated in the test medium and effects on Ni accumulation were determined through tissue analysis. An algorithm derived by relating equilibrium partitioning to the total available ligand was used to calculate the metal and major ion binding constants. These constants, valid for the range of test conditions explored, will be used in the development of a model for predicting toxicity of Ni to *H. azteca*.

Kinetics of Tributyltin Uptake and Depuration in *Hyalella azteca*: Implications for Experimental Design. A.J. Bartlett[1], D.G. Dixon[1], R.J. Maguire[2], S.P. Batchelor[2] and U. Borgmann[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Environment Canada, National Water Research Institute, Burlington, ON.

It is important to understand the behaviour of a contaminant in the organism in which it is being studied before designing toxicity bioassays. The purpose of this study was to answer the following questions about the kinetics of tributyltin (TBT) in the freshwater amphipod, *Hyalella azteca*: [1] how quickly are steady-state body concentrations reached, [2] is TBT accumulated primarily from the dissolved or particulate fraction, [3] do *H. azteca* depurate TBT, and [4] is there a significant effect of gut clearance on body concentrations of TBT.

Answers to the first two questions were obtained by exposing one set of *H. azteca* to spiked sediments, and suspending a second set of amphipods in cages above the sediment. The first set of animals was exposed to both the dissolved and particulate fractions of TBT, while the second set was exposed only to the dissolved fraction. Body concentrations of TBT were measured at different exposure times and fitted to an uptake curve to estimate the time at which steady state concentrations were reached. Differences in rate and extent of uptake from exposure to different fractions of TBT were also evaluated. To answer the last two questions, *H. azteca* were exposed to either water or sediment spiked with TBT for 2 weeks, and then transferred to clean water for varying durations over a 4 week period. Based on previous studies, decreases in TBT during the first 6 h were attributed to gut clearance. Body concentrations reached background levels within the 4 week period.

Use of Terrestrial Microcosms or Terrestrial Model Ecosystems to Assess the Fate and Effects of Chemicals and Biotechnology Products in the Canadian Context. M. Bombardier[1] and M. Smith[2]. [1]Environment Canada, Environmental Technology Centre, Ottawa, ON; [2]Department of Biology, Carleton University, Ottawa, ON.

The evaluation of the risks associated with the environmental release of chemicals and, more recently, biotechnology products requires a thorough understanding of their fate and effects in natural ecosystems. Terrestrial microcosms or terrestrial model ecosystems (TMEs) have great potential as risk assessment tools but, despite their recognized need and increasing use, there is no consensus within the scientific community about the most appropriate design and the methods for using it.

The aim of our program is first to conduct a comprehensive literature review on the state-of-the art of soil microcosm testing in ecology and in the ecotoxicological assessment of chemicals, and second to sponsor research which will lead to more standardized testing systems and measurement approaches. One immediate priority is to identify which TMs or TMEs should be used for testing the fate and effects of chemicals in soil ecosystems and further discuss effective use of soil microcosms as tools for studies on biotechnology products, in order to address some of the technical data requirements of various programs administered by Environment Canada. The following is a summary of the major conclusions, research gaps and recommendations from the review report, and an outline of a study where TM's are being used as the testing system for assessing the survival and persistence of four microorganisms listed on the CEPA (*Canadian Environmental Protection Act*) Domestic Substances List.

Controlled Toxicant Delivery: A Novel *in vivo* Approach for Embryotoxicity Testing. Y. Kiparissis, P. Akhtar, R.S. Brown and P.V. Hodson. School of Environmental Studies, Queen's University, Kingston, ON.

In conventional embryotoxicity assays to establish the toxic threshold of compounds the nominal rather than the actual concentrations are used. However, actual and nominal concentrations may differ by several orders of magnitude since parameters such as adsorption, volatilization or precipitation may cause rapid depletion of the test chemicals, especially for non-polar compounds (e.g., PAHs). In addition, nominal concentrations often exceed by far the solubility limits of the test compounds. In the present study, we present a reliable controlled delivery method that maintains the level of toxicants to test solutions at or below their solubility limits over an extended period of time. Polydimethylsiloxane (PDMS) films containing various levels of retene were deposited on one side of 20 mL vials and fertilized Japanese medaka (*Oryzias latipes*) eggs were placed on the other side. Films with retene at equilibrium provided aqueous solutions of 2 to 17 μg/L, which are below or at the solubility limit at 25°C.

Results from the PDMS film embryotoxicity assay were compared with results from a static non-renewal and a 24 h static renewal assay, in which the nominal concentrations ranged from 100 to 10,000 μg/L, and 10 to 1,000 μg/L respectively. All three embryotoxicity assays caused similar effects, with mortality and the prevalence of blue-sac disease (BSD) symptoms increasing in a dose-dependent manner. The median lethal concentrations (LC_{50}s) in the non- and 24 h renewal assays were estimated as 4,000 μg/L and 150 μg/L. In the same assays, the prevalence of BSD was significantly elevated at the 5,000 μg/L and 320 μg/L treatments, respectively. However, in the PDMS film assay, BSD and mortality occurred at retene's water solubility limits (i.e. 17 μg/L). Overall, these data indicate that the PDMS film assay is a very sensitive and a more realistic approach than the static assays to assess the embryotoxic potential of non-polar compounds.

HPLC Methods for Identification and Analysis of Polycyclic Aromatic Hydrocarbon Metabolites in Fish Tissue. R.S. Brown[1], S.P. Tabash[1], P.V. Hodson[2] and M.G. Ikonomou[3]. [1]Department of Chemistry, Queen's University, Kingston, ON; [2]School of Environmental Studies, Queen's University, Kingston, ON; [3]Department of Fisheries and Oceans, Institute of Ocean Sciences, Sidney, BC.

Polycyclic Aromatic Hydrocarbons (PAHs) are environmental pollutants that are known to be toxic to fish, and recent studies have indicated a particular toxic role for alkylated PAH compounds. As a result, there is now a need for developing methods for the analysis and identification of metabolites of alkyl-substituted PAHs. It is presumed that the toxicity is related to metabolism of these compounds in the liver by Mixed Function Oxygenases, including Cytochrome Oxidases. Model experiments to generate and characterize these metabolites have been developed in our labs.

Postmitochondrial supernatant (S9 fractions) from β naphthoflavone-induced liver tissue has been used for the formation of Phase I metabolites from methylpyrene and a series of alkylphenanthrene compounds. HPLC methods were able to separate metabolites and parent compound from other constituents in bile and liver homogenate, with detection by fluorescence, photodiode array, and mass spectrometry (MS). The methylpyrene experiments provided confirmation of methods to identify metabolites, since candidate metabolites were commercially available. The same methods were then applied to alkylphenanthrenes, where metabolites were not available separately as reference materials. In summary, metabolism of alkylPAH was dominated by oxidation of the alkyl-carbons on the molecule, perhaps accounting for dramatic differences in toxicity between these and "regular" PAH compounds. Further studies involving mass spectrometry to confirm the identity of metabolites and MS/MS to elucidate their structure will be discussed.

Alkyl Substitution of Phenanthrene Increases Potency for Cyp1a Induction and Toxicity to Fish. P.V. Hodson[1], Y. Kiparissis[1], X. Cai[1], T. Cross[1], S.P. Tabash[1], S. Blazeski[1], R.S. Brown[1], V. Snieckus[1] and C.D. Metcalfe[2]. [1]Queen's University, Kingston ON; [2]Trent University, Peterborough, ON.

The toxicity of Exxon Valdez oil to early life stage (ELS) of salmon and herring was correlated with concentrations of alkylphenanthrenes (alkylP) in interstitial water. We synthesized C_1 to C_4 alkylPs to test their CYP1A induction potency in trout and toxicity to ELS of medaka. Octanol-water partition coefficients were estimated by reverse-phase HPLC with solvent gradients to formulate structure activity relationships. Gram quantities of alkylPs in >98% purity were prepared by remote methylation using 5 steps, with 20 to 34% overall yields and minimal handling of toxic materials.

Toxicity to ELS increased in the order of P <1-methylP <2-ethylP <1,7-dimethylP <7-ethyl-1-methylP <7-isopropyl-1-methylP (retene); 2-pentylP was non-toxic at 1,000 µg/L. The rank order of potency for CYP1A induction was similar to the rank order for toxicity, and increased with the number, size and location of alkyl substituents; the low toxicity of 2-pentylP was likely due to steric hindrance. Medaka exposed to retene during sexual maturation showed impaired spermatogenesis, testis-ova, and fin erosion, similar to effects caused by MESA crude oil. AlkylP and Phase II metabolites were characterized in bile and liver homogenates by HPLC with uv-diode array, and fluorescence and mass spectrometric detection. Phase I metabolites were created by incubating alkylP with CYP1A enzymes from fish liver S9 fractions. Hydroxylation of alkyl side-chains in preference to ring hydroxylation might explain the differences in toxicity between P and alkyl-P.

New Methods for Assessing the Ecological Risk of PAH-Contaminated Sediments to Fish. A.E. Winchester, M. Windle, N.M. Fragoso, T. Cross and P.V. Hodson. Department of Biology, Queen's University, Kingston, ON.

Aquatic sediments act as sinks for many environmental contaminants, including polycyclic aromatic hydrocarbons (PAHs). In lab experiments, waterborne PAHs are bioavailable to fish and particularly toxic to their early life stages. *In situ,* exposure of caged juvenile rainbow trout to contaminated sediments demonstrated that PAHs were bioavailable to fish. A parallel lab experiment with the same sediments indicated that exposure of rainbow trout embryos caused increased rates of developmental abnormalities and mortality. The severity of effects increased in accordance with PAH concentrations. Is there any evidence that PAHs are toxic to ELS *in situ*? We adapted a method for testing the quality of Lake trout spawning shoals to assess the toxicity of contaminated sediments to rainbow trout *in situ*. Preliminary lab tests of the cages displayed low mortality due to cages alone and increased mortality in the presence of sediments.

Readiness of the Rainbow Trout Liver Hepatocyte Test for Standardization as an Acute Toxicity Method for Screening Effluents. J.A. Miller[1], R.P. Scroggins[2], F. Gagné[3] and C. Blaise[3]. [1]Miller Environmental Sciences Inc., Innisfil, ON; [2]Environment Canada, Environmental Technology Centre, Ottawa, ON; [3]Environment Canada, Centre Saint-Laurent, Montreal, QC.

In this test, freshly prepared hepatocytes from juvenile rainbow trout are exposed to liquid sample for 48 h at 15°C. After the exposure period, hepatocytes are collected for cell viability evaluations. Cell viability is determined either by the propidium iodide (PI) exclusion technique or the neutral red (NR) uptake inhibition technique. The PI method can be adapted to a microplate or cuvette fluorometer and the NR uptake test can be determined either spectrophotometrically or fluorometrically. In a recent study, trout primary hepatocytes were exposed to various industrial and municipal effluents for 48 h. Fingerling trout were also exposed to various effluents for 96 h using Environment Canada's rainbow trout acute lethality test.

The results showed that 33 out of 42 effluents were concordant in that the hepatocyte test was able to detect absence and presence of toxicity in effluents. This data suggests that there is 80%

concordant between the rainbow trout acute lethality and hepatocyte tests and therefore, high potential for the hepatocyte test to be an excellent effluent screening technique. In the non-concordant effluents, most samples indicated that the hepatocyte test identified toxicity although the trout acute lethality test did not respond. Although a number of procedural items will still have to be clarified, a recent assessment of the research behind the development of the hepatocyte test concluded that the procedure is ready for the standardization process that would lead to a national Environment Canada biological test method. The proposed national method will be described along with steps necessary to standardize this procedure.

The Duckweed Growth Inhibition Test Revisited - Data Acquisition by Image Analysis and New Applications in Risk Assessment of Soils. M. Eberius[1], I. Reuter[2], G. Mennicken[2] and I. Schuphan[2]. [1]LemnaTec GmbH, Schumanstr. 18, D-52146 Wuerselen, Germany; [2]Chair of Biology, Aachen University of Technology, 5, D-52056 Aachen, Germany.

Following the large number of national standards the duckweed growth inhibition test is now standardised by ISO for environmental samples. With a duration of 7 d the duckweed test is the fastest test with a higher aquatic plant. Duckweed is easy to culture in the lab and thus guarantees the continuous supply with high quality testing material over the entire year. Due to strictly vegetative growth a single clone may be used in different laboratories and allows high stability and comparability of the results. The ISO test guideline was especially developed for environmental samples to maximise robustness and minimise interference with non toxic matrix effects. As duckweed colonies are drifting on the surface of water, turbid or coloured samples do not interfere with growth just by their physical properties as long as a black bottom of the cultivation unit eliminates additional illumination due to reflection. A pH-value between 5 and 8 is no problem.

As all higher plants, duckweed may show different and complex reactions to different toxic substances. While some substances just reduce growth rate, other symptoms of toxicity observed are reduced size of fronds and more or less distinct patterns of chlorosis and necrosis. If toxicity and inhibition of growth should be quantified all these observations must be taken into consideration. This leads to high workload (manual counting of fronds) and methodological problems as quantification of dry weight and chlorophyll is destructive.

Recent advances in the technical development are now able to solve these problems and direct quantification of growth to a new quality of data acquisition. Using high- end image analysis, background objects are eliminated and each frond is recognised separately. This automatisation step provides quantitative data about frond number, frond size distribution and total frond area with a minimum of manual work.

The quantification of frond area has some important consequences for the results of the test. Total frond area is as sensitive or even more sensitive than frond number i.e., EC-calculations based on frond area generally result in minor EC-values than those based on frond number. The simple but important reason for this is the fact that besides frond number the size of each frond is generally reduced under toxic impact. The second important aspect is the problem of starting material. Especially for exponentially growing systems it is very important to inoculate all vessels as equal as possible. But starting with 10 to 16 fronds in two- or three- frond colonies inevitably leads to differences not in number but in area of the fronds.

Data show clearly that besides differences in illumination and general "biological variability" the total frond area of the inoculation material is a decisive factor for the coefficient of variation of the control at the end of the test. This affects test validity, investment of lots of time in accurate inoculation and the possibility to detect low toxicity by statistical means. If total frond area is measured, the differences in starting values are automatically integrated in the calculation of growth rate and inhibition. The displayed data show the results of a test with excellent inoculation (CV of frond area at the start 3.4%). But nevertheless the CV for growth rate of frond number is 3.1% (7.4 for final biomass of frond number) for 10 replicates of control at the end of the test. This may be reduced to 1.7% (CV growth rate) if total frond area is used instead. More often CV of starting area is in the range of 5 to 10% (not 3.1% as in this case) thus resulting in higher CV at the end of the test drastically reducing significance for low EC values. But these low EC-values become more important if the NOEC concept will be successively abandoned. The example describes that in many cases a high CV in duckweed tests is not a problem of the biological variability but a problem of inappropriate measurement methods for growth. The duckweed system has extraordinarily high precision, if calculation of inhibition is based on growth rate and frond area as intrinsic biological growth factors in full compliance with the ISO standard.

But image analysis adds additional value, if high quality colour imaging is used. Colour being a decisive parameter for the vitality of plants is difficult to quantify reproducibly by eye. Using image analysis colour may be classified by the user in different shades of green, yellow, brown or white. As a result all images are classified reproducibly thus displaying even small but significant shifts e.g., from medium to pale green (as very common for toxicants) or additional dark green area due to photosynthetic active herbicides. The results may be displayed at different levels of detail in dependence of concentration or frond size. Additionally colour classification can be used to count all fronds that are more than 50% green according to some testing standards.

Taking all information that is quantitatively available together provides a comprehensive image of the test performed at a minimum amount of workload. Quality control of tests and effective risk assessment based on a solid set of data adds additional value to customer and offers possibilities for later reanalysis without additional testing.

This innovative and efficient method to quantify the duckweed growth inhibition test was used to detect soil contaminations following ISO CD 20079. Soil elutriates were prepared at a soil :water weight ratio of 1:2 by overhead shaking for 16 h and centrifugation at 2,100 g for 30 min. No further filtration was made. *Lemna minor ST* was used as test species. While most of the tests were performed at the Technical University of Aachen/Germany in co-operation with LemnaTec/ Germany four of 15 soils were tested in an inter-laboratory comparison at ECT/Germany and UFZ Leipzig Halle/Germany. The project was part of a large German network project for the implementation of biotests in the regulative risk assessment of contaminated sites organised by IUCT Schmallenberg/Germany and funded by Deutsche Bundesstiftung Umwelt (DBU).

As one important result the Steinberg nutrient medium being far more concentrated than e.g., the modified SIS medium of OECD is very appropriate for effluents or soil elutriates. Stimulation of growth relative to the medium control is prevented effectively to maximize sensitivity of the test system. As the matrix effects are minimized, all tests showed that no non-contaminated soil of similar structure is necessary as reference. This is of high importance for a valid and effective test performance and the subsequent risk assessment as generally no reference soil is available.

Tests with pure substances showed the duckweed test to be highly sensitive to a wide variety of contaminants like mineral oil products, heavy metals and e.g., explosives and their by- or degradation products.

All tested soils with chemically defined contamination were detected by the duckweed test as contaminated, except one soil from a gaswork plant which was classified as "suspected contamination." Some soils not showing significant effect later on revealed to be in the rage of environmental background contamination. One of two soils remediated according to German laws revealed to be highly phytotoxic. On other soil without any contamination getting obvious by standard chemical analysis could be shown to be highly toxic for higher plants.

Taking all different test systems of the whole project together it was clearly shown that only a test battery is able to perform effective hazard assessment. Duckweed revealed to be one of the most sensitive organisms being able to detect a wide range of different xenobiotics from heavy metals to organics like explosives, PAH, mineral oil products even at low but relevant concentrations.

For this reason the duckweed test was strongly recommended to add to the actual test battery after standardisation is finished. Compared to algae, duckweed has a much higher potential to detect contaminated soil. Being a one week test the duckweed test is, together with *Vibrio fischeri* an excellent first step in general soil contamination classification or more special to assess risk for groundwater or surface water. Plant growth tests and reproductive tests in the soil (earthworms, springtails..) with a duration of some weeks can later on give important additional sensitive information about soil as potential habitat for higher organisms.

It is important to emphasize the high sensitivity of higher plant testing either in the aquatic or terrestrial field. Their sensitivity has been underestimated for a long time but now new test systems and advances in technical measurement of growth give additional impact to strengthen research on this field in future.

The ISO test design was shown to give highly reproducible results both from the perspective of biology and regulation as a state of the art synthesis of ecotoxicology, information technology, statistics and documentation. Therefore the duckweed test is able to fill the gap of higher plants in many bio test batteries.

For a literature list, more information and update on duckweed testing, standardisation and results of the soil risk assessment project please contact: Matthias Eberius, LemnaTec GmbH, Schumanstr. 18, D-52146 Wuerselen, Germany, Phone: 0049 2405 412615, Fax 0049 2405 412626, *eberius@lemnatec.de, www.lemnatec.com*

The Use of TIEs to Characterize Toxic Constituents in a Diamond Mine Effluent. S.J. Crocquet de Rosemond[1], K. Liber[1], D. Waite[2] and S. Harbicht[3]. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; [2]Environment Canada, Environmental Conservation Branch, Regina, SK; [3]Environment Canada, Environmental Protection Branch, Yellowknife, NT.

Diamonds are found in kimberlite deposits and extracted mechanically using chemical-free processes that reduce the ore to fine particles (<0.5 mm). The processed effluent consists of approximately 40% kimberlite fines and 60% water that has been used during the extraction. Coagulating and flocculating polymers are added to the effluent to facilitate settling of the fines in the containment facility, Long Lake. Baseline toxicity tests using *Ceriodaphnia dubia* indicated that the effluent is chronically toxic. A series of Phase I Toxicity Identification Evaluations (TIEs) have been performed in an attempt to classify toxic components of this effluent.

Chronic serial dilution toxicity tests indicated that filtered effluent significantly affected survival at concentrations of approximately 50%, and reproduction at concentrations as low as 12.5%. Toxicity of the effluent to *C. dubia* was not significantly reduced through aeration, solid phase extraction using a C-18 column, nor through the additions of either sodium thiosulphate or EDTA. Graduated pH adjustments of the effluent to pH 6.5 significantly reduced toxicity but did not eliminate toxicity. Further pH manipulations of unfiltered effluent to pH 3.0 completely eliminated toxicity. Toxicity testing of the polymers determined that the coagulating polymer is acutely toxic to *C. dubia* at low concentrations; the 48 h LC_{50} was 0.32 mg/L, while the flocculating polymer was relatively nontoxic. The loss of effluent toxicity at low pH may be due to the stabilization of the coagulating polymer in conjunction with hydrolysis of kimberlite minerals.

A Toxicity Evaluation of Weathered PHC Soils Following Biotreatment Versus CCME PHC Standards. S.M. Murphy and J.W.A. Charrois. Komex International Ltd., Edmonton, AB.

The Canadian Council of Ministers of the Environment (CCME) recently developed (June 2000) standards for petroleum hydrocarbon (PHC) contamination in soil. These standards are based partly on toxicity testing, conducted with various hydrocarbon fractions freshly added to pristine soils. Bioavailability of fresh PHC may not be representative of aged PHC contamination in soil. A battery of toxicity assays was employed to evaluate: [1] the toxicity of residual PHC in weathered biotreated soils collected from four upstream oil and gas facilities and a pristine soil, and [2] the new CCME PHC standards versus the observed toxicity of weathered hydrocarbons in soil. Soils were analyzed for PHC, PAHs, pH, and particle size. Toxicity bioassays included: Microtox®; seed germination (*Raphanus sativus*, radish and *Avena sativa*, oats); as well as acute (14 d) and subchronic (16 weeks) exposures of earthworms (*Eisenia fetida*). After the subchronic exposures were complete, earthworm tissues were analyzed for hydrocarbons and hydrocarbon metabolites.

All soils contained PHC in excess of CCME standards for various soil types, land uses and exposure pathways. Microtox® results indicated that all soil extracts were non-toxic. Seed germination results indicated >90% of seeds (both species) germinated within four days, in all soils. Earthworm survival was 100% for both acute and subchronic exposures. Further, trimethylnaphthalene and two isomers of ethoxyphenyl acetone were quantified in earthworm tissues. These results suggest the risk of weathered PHC in bioremediated soils may not be captured by current CCME PHC standards.

Contaminant Dynamics in Limnological Systems

Session Co-chairs: G.D. Haffner, A. Salki and M. Paterson

Variation in Mercury Concentrations in Predatory Fish in the Northwest Territories and Northern Alberta/Saskatchewan: The Role of Fish Length, Age, Food Web Structure, Water

Chemistry, and Watershed Size. M.S. Evans[1], W.L. Lockhart[2], G. Low[3], D.C.G. Muir[4], K.A. Kidd[2], G. Stern[2], J. Delaronde[2], S.J. Guildord[5], W. Strachan[4], D.M. Whittle[6] and X. Wang[4]. [1]Environment Canada, National Water Research Institute, Saskatoon, SK; [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; [3]Department of Fisheries and Oceans, Hay River, NT; [4]Environment Canada, National Water Research Institute, Burlington, ON; [5]Department of Biology, University of Waterloo, Waterloo, ON; [6]Department of Fisheries and Oceans, Burlington, ON.

Monitoring programs conducted in the Mackenzie River Basin, NT, discovered that predatory fish in many lakes had Hg concentrations that exceeded the 0.5 mg/kg guideline established for the commercial sale of fish, and the 0.2 mg/kg guideline established for frequent consumers of fish. As a consequence, consumption advisories were issued for these lakes: with further monitoring additional advisories are being issued. In contrast, Hg concentrations in predatory fish in northern Alberta and Saskatchewan lakes tend to be lower. Here we report the results of studies investigating Hg levels in predatory fish in both locations.

In the NT, elevated Hg concentrations in predatory fish are not related to high Hg concentrations in water and sediments or to very low pH although watershed size and water color are of some importance. Hg levels are more strongly related to trophic feeding, fish size and fish age. Similar factors are important in lakes in northern Alberta and Saskatchewan. The most striking difference between predatory fish in the NT and northern Alberta/Saskatchewan, in addition to their greater Hg concentrations, is their greater age.

Factors Affecting Mercury Concentrations in Top Predator Fish in Northern Lakes across Canada. K.A. Kidd[1], M.R. Anderson[2], M.S. Evans[3], W.L. Fairchild[4], S.J. Guildford[5], D.C.G. Muir[6], J.F. Payne[2], D.M. Whittle[7] and X. Wang[6]. [1]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg MB; [2]Department of Fisheries and Oceans, St. John's, NF; [3]Environment Canada, National Water Research Institute, Saskatoon SK; [4]Department of Fisheries and Oceans, Moncton NB; [5]Department of Biology, University of Waterloo, Waterloo ON; [6]Environment Canada, National Water Research Institute, Burlington ON; [7]Department of Fisheries and Oceans, Burlington, ON.

Studies of lakes within a limited geographical area have demonstrated that the concentrations of Hg in top predators are related to size, age, trophic position and inherent lake characteristics. In this study, muscle of brook trout, pike, walleye and lake trout (n=5 to 20) collected in 1999 to 2001 from 30 lakes (9 in Labrador, 5 in New Brunswick, 5 in southern Ontario and northern New York state, 5 lakes in northwestern Ontario, 2 in Alberta and 4 in Saskatchewan), as well as food web samples, were analysed for Hg and stable isotopes of C and N (to determine trophic positioning). The main objective was to determine the physical, chemical and biological factors that predict Hg concentrations in fish from a wide range of oligotrophic and mesotrophic lakes.

Hg levels ranged from 0.232 to 1.1 mg/kg wet wt in lake trout muscle and from 0.22 to 0.66 mg/kg wet wt in pike with highest levels in Labrador. Mean Hg levels (length adjusted) were significantly higher in fish (lake trout, walleye and brook trout) in eastern Canadian lakes and were significantly correlated with longitude (r^2=0.42) and weakly, inversely, related to lake size. Relationships between Hg concentrations in these top predators and age and trophic positioning, as well as growth/feeding rates, size spectrum and nutrient status of microplankton, overall trophic status of the lake productivity, littoral zone feeding, and catchment area, were also examined.

Spatial Trends and Trophic Transfer of POPs and Current Use Pesticides in Fish across Canada: An Overview. D.C.G. Muir[1], M.R. Anderson[2], K.G. Drouillard[3], M.S. Evans[4], A.T. Fisk[1], W.L. Fairchild[5], S.J. Guildford[6], G.D. Haffner[3], K.A. Kidd[7], J.F. Payne[2], D.M. Whittle[8] and X. Wang[1]. [1]Environment Canada, National Water Research Institute, Burlington ON; [2]Department of Fisheries and Oceans, St. John's, NF; [3]Great Lakes Institute for Environmental Research, University of Windsor, Windsor ON; [4]Environment Canada, National Water Research Institute, Saskatoon SK; [5]Department of Fisheries and Oceans, Moncton, NB; [6]Department of Biology, University of Waterloo, Waterloo ON; [7]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg MB; [8]Department of Fisheries and Oceans, Burlington, ON.

Although measurements of PCBs and other persistent organics (POPs) in freshwater fish have been conducted for 30 years in Canada there are relatively few recent datasets that can be used to assess geographical trends in contaminants and factors influencing variation in levels among locations. In this presentation we will examine recent geographic/spatial trends in top predator fish (burbot, lake and brook trout and walleye) from across Canada. Our spatial trend data for lake and brook trout are derived from studies currently underway in lakes in Labrador, New Brunswick, southern Ontario and northern New York State, northwestern Ontario, Saskatchewan, and Alberta. Whole fish (or liver of burbot) were analysed for PCBs, persistent organochlorines and current use pesticides (endosulfan and lindane) as well as stable isotopes of C and N (to determine trophic positioning). Concentrations of ΣPCBs, toxaphene and ΣDDT in food webs of each lake were strongly correlated with δ15N in each sample. Effects of food chain and trophic status of the lake as well as to lipid content, size, and age of the fish are also being examined.

Using Trophic Transfer Efficiencies to Evaluate Site-Specific Differences in Chemical Bioaccumulation Potentials along the Huron-Erie Corridor and in Oligotrophic and Mesotrophic Lakes of Southern Ontario. K.G. Drouillard[1], D.C.G. Muir[2], D.M. Whittle[3], M.J. Keir[3] and G.D. Haffner[1]. [1]Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON; [2]Environment Canada, National Water Research Institute, Burlington, ON; [3]Department of Fisheries and Oceans, Burlington, ON.

A biological monitoring program was established to determine organochlorine trophodynamics along the Huron-Erie corridor (Lake St. Clair, Detroit River, western basin of Lake Erie) and in a mesotrophic and an oligotrophic lake of Southern Ontario, Canada. Representative organisms were sampled to include benthos (pooled samples), benthic feeding fish, forage fish, pelagic fish and large piscivorous fish. Trophic transfer efficiencies were determined for individual chemicals using the slope of the regression between log chemical concentrations in organisms vs. trophic status. Trophic transfer efficiencies provide an index of biomagnification potentials within the sampled system.

Trophic transfer efficiencies for different organochlorines exhibited an increasing tend with increasing chemical K_{ow} up to a log K_{ow} of 6.5 after which an asymptotic value was reached. The trophic transfer efficiency of Hg was similar to the trophic transfer efficiencies of high K_{ow} organochlorines. The magnitude of trophic transfer efficiencies across different locations varied and was most elevated in riverine samples, although the pattern of trophic transfer efficiencies across chemicals was consistent among sites. Site to site differences in trophic transfer efficiencies and biomagnification factors are related to differences in energy flow and feeding relationships of the sampled systems.

Assessing Herring Gull Egg Viability on the Great Lakes. C.E. Hebert, P. Mineau, J.L. Shutt and K. Williams. Environment Canada, Canadian Wildlife Service, Hull, QC.

Contaminant-induced early embryonic mortality was an important factor contributing to the low reproductive success observed in Great Lakes herring gulls during the 1970s. However, systematic surveys of egg viability have not been conducted because of the difficulty in assessing this endpoint in the field using herring gull eggs. To overcome this difficulty an electronic device was constructed to evaluate embryonic viability using non-destructive means. Combined with contaminant analysis, this endpoint provides a potential tool to assess geographic differences and temporal changes in contaminant exposure/effects. Effects-based measures of temporal change in contaminant impacts on wildlife are currently limited and would be useful in evaluating our progress towards achieving the virtual elimination of persistent contaminants in the Great Lakes.

In 2001, field surveys were conducted on herring gull colonies in the Lake Huron-Lake Erie Corridor to assess whether there were geographic differences in egg viability. Colonies on the western basin of Lake Erie (20%) and the Detroit River (17%) had higher proportions of dead eggs than did a reference colony on Lake Huron (5%). These preliminary results indicate that there are significant differences in egg viability among colonies and these differences are consistent with the degree to which these areas are contaminated with organochlorines. However, more research is required to demonstrate a chemical etiology for the observed differences in egg viability.

Evidence for Recent Environmental Changes in Lake Winnipeg. W.L. Lockhart, A. Salki, H.J. Kling and M.P. Stainton. Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

Lake Winnipeg is the 10th largest freshwater lake in the world. It drains a basin approximately 40 times its own area. With approximately 5.5 million people and several times that number of farm livestock, it is not surprising that Lake Winnipeg is showing signs of overloading. The human population is relatively stable but the livestock population has been growing dramatically. In spite of its importance to the history, culture and economics of Manitoba, Lake Winnipeg has been the subject of surprisingly little scientific study.

There have been infrequent survey cruises of the lake, beginning as early as 1929 and extending to 1999; these have provided a series of "snapshots" of the condition of the lake at those times. The results have indicated that the lake is undergoing several changes, the most troublesome of which appear to be increasing concentrations of nutrients, increasing frequency of "blue-green" algae, introductions of exotic species, and loadings with an array of trace chemicals.

While these changes have been occurring, the inflows and outflows have been controlled in order to store water for production of hydroelectricity on the Nelson River. These hydrologic changes appear to have affected the transparency of the North Basin, presumably by trapping particulate matter above the Grand Rapids dam. With greater transparency and growing nutrient concentrations, extensive algal blooms, notably cyanophytes, have developed. At the same time, the lake has been invaded by exotic species of fish, notably rainbow smelt, and the invertebrate *Eubosmina coregoni.* (Zebra mussels have not been found in the lake to date). Other chemicals like Hg and PCBs also enter the lake; indeed levels of Hg in the fish forced cancellation of fishing for a short period. Taken together, the lake presents a complex picture that is not currently understood. The challenge for regulators is to limit inputs of substances and species and regulate flows so that the lake ecosystem is sustained. But in order to do that, an understanding of how the lake functions must be developed.

Improved Detection of Microcystin-LR using HPLC with UV detection in samples from Lake Winnipeg and Lake of the Woods. C.R. Herbert[1], H.J. Kling[1] and M.P. Stainton[2]. [1]Algal Taxonomy and Ecology Inc., Winnipeg, MB; [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

Lake Winnipeg, the largest freshwater body in Manitoba, supports a large commercial fishery as well as being a source of drinking water to communities surrounding and downstream from it. Although few complete limnological studies have been conducted on the lake, recent surveys of algal species, chlorophyll and nutrients point toward an increased eutrophication and dominance by cyanoprokaryotes. Large blooms of *Microcystis, Anabaena, and Aphanizomenon,* known producers of the toxin microcystin are now dominant in the lake during the late summer.

Lake of the Woods is a large international lake situated primarily in Northwestern Ontario and extending into Minnesota. Besides being an important recreational area it commonly supplies drinking and household use water to the cottage owners located on its shores. Blooms containing *Aphanizomenon*, *Anabaena* and *Microcystis* have also been observed on this lake. Using HPLC with UV detection, following a method modified from Carmichael and Brittain (unpubl. data 2000), we analyzed samples taken from both lakes in 1999 and 2000 for Microcystin. We also examined methods to accurately and rapidly extract and purify the samples before analyzing them with HPLC by utilizing a variety of Solid Phase Extraction columns (SPE) to reduce the amount of interference in the chromatogram by other pigments.

Role of Disturbance in Lake Evolution and Littoral Development and Implications to Restorations. N. Munteanu and G.P. Thomas. G3 Consulting Ltd., Burnaby, BC.

Abstract

Disturbance (climatic, morphometric or edaphic phenomena that change minimal ecosystem structure) has long been recognized as having important effects on structure and dynamics of ecological communities. Emphasis has recently been placed on considering certain disturbances to be natural processes occurring at different spatial and temporal scales. Though short-term, detrimental effects on fisheries resources of such disturbances as mass loading are well documented, salmonids and other native fishes have co-existed with naturally occurring landslides for millennia post-glaciation, especially in areas of steep, unstable terrain. This paper presents a discussion of lake succession and long-term effects on aquatic ecosystems of sediment loading and nutrient input from natural disturbances (e.g., debris torrents, landslides and rock falls).

Drawing on evidence from long-term studies of landslides and of artificial reefs, the concept of disturbance and change as an integral and necessary part of lake-ecosystem structure, function and evolution is discussed. The argument relies on paradigms of stochastic change and elastic stability propounded by researchers who advocate a "nature evolving" view of ecological succession and the role of disturbance in interrelated ecosystem functions (exploitation, conservation, creative destruction and mobilization). The present status of ecosystem management and restoration are reviewed in this context. The need to expand the frame of reference of ecosystem restoration is discussed, together with new approaches being advocated by ecosystem managers who stress sustainability of ecological integrity (including structural, functional and landscape components).

Introduction

Short-term, detrimental effects of such disturbances as mass loading on aquatic systems is well documented. Salmonids and other native North Pacific fishes have, however, coexisted with naturally occurring landslides for millennia since the most recent glaciation, especially in areas of steep and unstable terrain. Infrastructure construction and maintenance activities (e.g., roads and railways) can considerably increase the severity and frequency of landslides. Northcote and Northcote (1996) and Northcote (1997) discussed effects of lake-edge alteration (e.g., Lake Constance and Lago Maggiore) by such anthropogenic changes as placement of cement and riprap.

Though human action to intentionally deposit deleterious substances in a water body is considered a violation of the Canadian federal *Fisheries Act*, nutrient loading of lakes from landslides and related events that deposit sediment are natural processes within the larger scope of ecosystem function and evolution. This paper compares long-term benefits to aquatic ecosystems of sediment loading and nutrient input from natural disturbances (e.g., debris torrents, landslides and rock falls) with perceived short- and long-term adverse impacts on fisheries resources.

Disturbance and Change in Lake Ecosystems

Originating primarily from geologic processes, concave lake basins have a natural tendency to fill with sediments. Lake ecosystems exhibit dynamic and stochastic properties in their natural progression toward eutrophication. Site-specific characteristics determine an evolutionary path unique to each system. Climatic, morphometric and edaphic phenomena continually influence a lake, making single-event changes or ones that are cyclical or recur in the short- or long-term. Such phenomena, or disturbances, are inherent to ecosystem behaviour (Holling 1987, Minns *et al.* 1996) and can be anticipated. Pickett *et al.* (1989) defined disturbance as change in the minimal structure (of the ecosystem) caused by a factor external to the level of interest. "External" refers to any action that originates outside the unit (ecosystem) in question.

Landscapes, whether terrestrial or aquatic, are a mosaic of patches, the components of pattern (Urban *et al.* 1987). Natural agents of pattern formation are interwoven in landscape development and comprise disturbances, biotic processes and environmental constraints (Levin 1978). Disturbance has long been recognized as an important factor affecting community structure and dynamics, and ecologists are increasingly considering some types of disturbance to be natural processes that occur at different spatial and temporal scales. Disturbances vary in spatial extent, recurrence interval, and intensity (Pickett and White 1985).

The significance of scale of disturbance, whether in time, intensity, or spatial extent, is borne out by the example of fire in a terrestrial habitat (Pickett *et al.* 1989). Single fires can disturb individuals or entire populations without necessarily altering a community. Altering a community requires a change in fire regime (e.g., an alteration in frequency, extent or intensity of fires).

Concepts of Change

Holling (1977, 1987) discussed a sequence of three distinct perceptions ecologists have of how nature behaves and changes: [1] an equilibrium-centred, "nature constant" view, [2] a dynamic view of multiple equilibria that are "nature engineered" or "nature resilient," and [3] a "nature evolving" view. The equilibrium-centred view emphasizes constancy over time, not only of behaviour, but also of spatial homogeneity and linear causation. By contrast, the multiple equilibria view emphasizes the existence of a number of stability regions and the role of instability in maintaining resilience of ecological systems. In the "nature resilient" variant, instability may be seen as maintaining resilience of ecological systems and emphasizes variability, spatial heterogeneity and nonlinear causation (Holling 1973); structure and general patterns of behaviour are maintained through instability (e.g., disturbance). Holling and others advocate the "nature evolving" view of ecosystem behaviour, which describes ecosystems as undergoing sharp, discontinuous changes that are internally organized and balanced, but have shifting variability. The stress is placed on evolution of ecosystems, highlighting organizational change and unforeseen phenomena incurred by such change, the premise being that a variety of genetic, competitive and behavioral processes maintain balances with shifting variability.

Continual influences of climate and physicochemical phenomena may cause short- and long-term changes and cycles in a lake. Such "disturbances" are inherent to lacustrine ecosystems and would be anticipated. Holling's "nature evolving" ecosystem cycles through several stages applicable to lakes: exploitation (pioneer, opportunist, r-strategy); conservation (climax, consolidation, K-strategy; e.g., old growth forest); creative destruction (fires, storms, pests, senescence); and mobilization (nutrients and energy).

Through succession from the exploitation phase, natural forces of conservation lead to vulnerable systems (e.g., old growth forests), as stabilizing factors lose strength and the system evolves from having few inter-relationships to having many. The result is often an abrupt change that both destroys systems and creates opportunity, which Holling termed creative destruction after Schumpeter's (1950) economic model. Mobilization of bound, stored "capital" through such physicochemical and biological processes as decomposition and mineralization completes the dynamic cycle of functional ecosystems.

Current Management and Regulation of Nature

Examples of current management practices that can be viewed as "weak" experiments in a "nature constant" view include control of spruce budworm populations in eastern Canada using insecticides, suppression of forest fires in national parks of the United States, and protection and enhancement of salmon spawning on the west coast of North America. Such policies, when implemented successfully, reduced the probability of a socially or economically undesirable event and met short-term objectives. Long-term ecosystem changes, however, were often unforeseen and deleterious. Spraying of budworm delayed tree mortality and led to incipient infestations spread over large areas; fire suppression led to unnatural accumulation of fuel, changes in forest structure, and potential for wildfires of far greater devastation and cost; and salmon protection and enhancement sustained a local fishing industry but altered stock composition, with consequences yet unknown as the fishing industry was left precariously dependent on a few enhanced stocks that are vulnerable to collapse. Typically, biophysical environments have become more fragile and more dependent on vigilance and error-free management. These examples support the conclusion that the equilibrium-centred "nature constant" view applied by many managers and regulators may be inconsistent, and illustrate the important role of disturbance in maintaining ecosystem integrity.

Theories of evolution are not predictive, but rather retrospective and historical. An example is the appearance of plant life, which profoundly changed the global atmosphere. Another is the appearance of homeotherms (warm-blooded animals), which elicited a wave of evolutionary change.

The Littoral Zone

Littoral zones of lakes are peripheral shoreline shallows that are subject to fluctuating temperatures and erosion through wave action and grinding of ice (Cole 1975). The littoral zone constitutes the interface between the terrestrial portion of the drainage basin and open water, and is usually taken as extending to the maximum depth of submerged rooted and adnate macrophytes (Wetzel 1983). The slope and extent of a littoral region depends on the geomorphology of the basin and rates of sedimentation.

Littoral areas provide habitat to a diverse assemblage of biota and include colonizing surfaces for periphyton, benthic invertebrate communities and macrophytes, and foraging, rearing and cover habitat for fish and other larger biota. Littoral flora constitute a centre of synthesis of organic matter that contributes significantly to the productivity of lakes and the regulation of metabolism of the entire lake ecosystem (Wetzel, 1983). The littoral zone fish fauna plays an important role in freshwater lake ecosystems (Northcote 1988). Fish abundance is typically greater in the littoral zone than in other zones and may constitute the bulk of the total fish community (Keast and Harker 1977, Werner *et al.* 1977). Habitat and food resources tend to be more diverse in the littoral zone, leading to complex relationships with the fish community.

Effects of Landslides and Mass Wasting

Landslides have occurred in evolving lacustrine systems for millennia and fish and other biota in lakes have adapted and co-evolved along with these natural disturbances. Increased sedimentation resulting from river runoff, erosion and landslides may alter the submerged environment by causing burial, scour and increased turbidity. Fine debris may smother alevins. As they are incapable of movement, algae and sessile invertebrates are the most affected by sedimentation (Menge and Sutherland 1987), in turn affecting fish populations that forage in littoral zones, the most likely habitat region affected by slides. Suspended sediments may reduce available light for plants; scour may damage or kill plants and sessile invertebrates; and sediment deposition may limit available oxygen and space for settlement (Konar and Roberts 1996).

It may also be argued that natural landslides perform a necessary role in lake evolution. As littoral zones of a lake evolve along with the entire lake, a young lake having steep sides and small littoral zone would have a natural tendency to evolve toward a less steep-sided lake with more extensive littoral areas, ultimately providing more habitat for young fish and benthic life.

Aquatic scientists recognize sedimentation as integral to both marine and freshwater aquatic systems, transporting critical nutrient sources to the aquatic environment (Stow and Chang 1987, Bavestrello *et al.* 1991). For instance, in a study of Monterey Canyon, California, Okey (1997) determined that large autumn and winter storms disrupt and remove sediment and macrofaunal assemblages, but also import new resources and re-oxygenate habitat. Within four months of a documented landslide, researchers found no difference between macrobenthic communities of slumped and intact areas.

Ecological information derived from the marine environment by Hecker (1982) suggested that landslides (particularly submarine slides) destroy existing communities, make new space available, select for mobile species over sedentary ones, and transport organically rich sediment to deep water habitats. Other researchers have studied the implications on marine aquatic environments of mass wasting on energy and nutrient inputs, including elevated abundance of benthic fauna (Griggs *et al.* 1969, McHough *et al.* 1992).

Artificial Reefs and Littoral Development

The role of material-loading in development of littoral areas of a lake (shallows) has been successfully applied when restoring aquatic habitat by creating artificial reefs. Techniques employed to increase freshwater habitat availability, improve spawning substrate, and attract fish have varied from judicious placement of large boulders, root wads or felled trees to construction and placement of elaborate artificial reefs (Prince and Maughan 1978, McGurrin *et al.* 1989). Ambrose and Anderson (1990), when studying the effect on the surrounding marine community following placement of an artificial reef, demonstrated that the reef enhanced densities of some organisms and depressed others, the overall effect on the surrounding infauna being limited to a small area near the structure. The study also acknowledged that, at the very least, the reef destroys organisms buried underneath the structure as would a natural slide.

Fish attraction structures may provide opportunities for fishery enhancement and mitigation (Hueckel *et al.* 1989, Foster *et al.* 1994) by replacing lost habitat. Invertebrates employ underwater wood and debris as attachment sites and attract fish (Dudley and Anderson 1982, Wallace and Benke 1984). Submerged logs and debris have long been known to attract fish by providing protective cover and indirect environmental benefits of additional littoral complexing.

Options for Restoration and Management

Littoral vegetation increases and decreases naturally. Great storms redistribute substrates, nutrients and species, and each event initiates rejuvenation and recovery of habitats and communities. These perspectives and concepts are rarely acknowledged or considered during management, restoration or regulatory processes.

Regarding the ability to assess dynamics of fish populations and flux of nutrients in lakes and rivers, Allen *et al.* (1993) emphasized three perspectives: structure, function, and landscape. A structural perspective considers the species and communities that constitute living elements of the ecosystem. A functional perspective stresses movements of materials within and through ecosystems (e.g., energy, nutrients, water and sediment). A landscape perspective recognizes spatial and temporal elements and relationships among structural and functional features. Superimposed on these perspectives are nested hierarchies of organization, ranging from individual to population assemblage, and from ecosystem levels to land- and water-scapes (Picket *et al.* 1989, Minns *et al.* 1996).

Citing Holling's 1992 model of ecosystem spatiotemporal structure, Minns *et al.* (1996) used a nested hierarchy of spatial-temporal domains to describe the way populations exploit their environment. Home range, for instance, depended on size of the fish (Minns 1995) and population area depended on population size and lifestyle. Minns *et al.* (1996) then superimposed on this population-scale template various events and phenomena that occur and influence success of the population. They suggested that, "The scale of habitat interventions (e.g., spawning habitat change) remains localized both spatially and temporally; while events such as phosphorus or contaminant loading are system-wide and may have long-lasting implications."

Holling (1987) illustrated how structural and functional elements of ecosystems are linked by cyclical changes in capital, amounts of energy and nutrients sequestered in biomass, and degrees of ecological complexity and connectivity. Natural variability in nature confounds efforts to detect ecosystem responses to changes, including human interventions such as restoration activities (Minns *et al.* 1996). According to Minns *et al.* (1996), fisheries ecology and restoration practices have focussed largely on the exploitation-conservation part of nature. Unexploited, higher trophic levels accumulate biomass and complexity. Exploitation reduces capital and often genetic or community complexity. Holling's (1987) synthesis demonstrated how the frame of reference and scope of ecosystem restoration requires expansion to include concepts of creative destruction and mobilization phases of natural cycles. New approaches advocated by ecosystem managers stress sustaining ecological integrity through a broader scope and over a longer term than current practice.

Summary and Conclusions

Observations by Minns *et al.* (1996) and others (Griggs *et al.* 1969, Prince and Maughan 1978, Hecker 1982, Desrosiers *et al.* 1984, McHough *et al.* 1992, Okey 1997) raise the question of long-term system benefits of natural sediment loading and nutrient input from natural disturbances versus perceived short-term impact on fisheries resources identified in naturally occurring debris torrents, landslides, and rock falls.

It has been argued that landslides are natural and that human intervention in "preventing materials entering a system or removing material from the watershed," as recommended by Fisheries and Oceans Canada, may compromise ecosystem integrity over the long term.

Ecosystem management and restoration has largely followed a reactive approach, often targetting structural elements and assuming constancy of variables, due to insufficient knowledge, limited knowledge of overall ecosystem processes, and long-term implications. Such an approach is based on an imperfect equilibrium-centred "nature constant" perspective. New approaches are being advocated by ecosystem managers who stress sustainability of ecological integrity (including structural, functional and landscape components).

Landslide processes generally obey natural laws. It may be argued that, while certain human activities that exacerbate the frequency and severity of landslides may prove detrimental, human interference to prevent materials entering a system or remove material from a watershed may present greater long-term problems to ecosystem integrity than a lack of action.

Rapport (1989), Minns (1995) and others have suggested that a more holistic, integrative perspective to ecosystem management and restoration is needed, analogous to a linked mind-body view of human health (Minns *et al.* 1996). The AMOEBA-approach to ecosystem management (Brink *et al.* 1991; cited by Minns *et al.* 1996) represents such an approach with a broader scope and longer-term than current practice in most ecosystem management and restoration efforts (Minns *et al.* 1996). This broader perspective may be a viable approach and should be considered as part of regulatory protocols.

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Due Diligence and Litigation: Science in the Courtroom

Session Chair: D. Birkholz

Science and The Law. Judge L. Wenden. Alberta Provincial Court.

The ever increasing use of science in environmental disputes, whether it be in the courtroom or before tribunals has resulted in confusion concerning the roles and expectations of all the parties involved. Each party has different perceptions of their role, and the role of the other participants.

The presentation will focus on some of these, and the recurring problems that it causes.

Horror Stories in Environmental Law: It is only your Reputation! S. McRory. Alberta Justice, Special Prosecutions, Edmonton, AB.

Examples of how essentially good corporate citizens have found environmental prosecution turn into their worst nightmare will be presented. The reasons are many but usually include: a lack of realization of the inherent dangers, misconceptions and misunderstanding of their role in the legal system.

Who Needs a Lawyer? How Effective Legal Representation can Save Time, Money, and Reputations. S. Thomas. Owen and Company, Edmonton, AB.

Disaster strikes - you have an environmental incident. What should you do? Appearing as a witness before Boards and Tribunals - good science, bad science, and the ramifications. Contaminated lands - can you pass of the problem by selling the property?

Effective Presentation of Evidence. K. Short. TrialTec Consulting Inc., Edmonton, AB.

Effective means of presenting evidence using audio-visual aids will be discussed. Some exciting cases in which this approach has been applied will be demonstrated.

Aquatic Toxicology in Court: Is Routine Testing Good Enough for Legal Samples? G.R. Craig. Gordon Craig & Associates, Bolton, ON.

The level of quality assurance required to support testing of samples in contemplation of litigation will be discussed. Credentials of expert witnesses as well as the importance of laboratory accreditation will also be examined.

Going Fishing in Turbid Waters: How the *Fisheries Act* Might Catch You. J. Stefaniuk. Thompson, Dorfmann, Sweatman, Winnipeg, MB.

What constitutes an offense under the pollution and habitat provisions of the *Fisheries Act*? Why should I care? How can I protect myself and my company? What does the Crown need to prove? Why should I worry about environmental "bounty hunters"? Find out the answers and more.

Boreal and Arctic Ecosystems

Session Co-chairs: W.L. Lockhart and G. Stern

PCB and Organochlorine Biotransformation and Methyl Sulfone and Phenolic Metabolites in Arctic Marine Biota. R.J. Letcher[1], C.D. Sandau[2] and R.J. Norstrom[2]. [1]Great Lakes Institute

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Methyl sulfone ($MeSO_2$) PCB and DDE metabolites are persistent in biota due to their low reactivity, and lipophilic and bioaccumulative properties. In contrast, hydroxyl (OH) PCB and other metabolites of phenolic organohalogen substances (OHS) have been identified in blood are retained via protein binding. The growing body of reports on specifically $MeSO_2$-PCB and –DDE and OH-PCB metabolites in biota indicates that their formation is a common phenomenon across species of mammals (including humans), birds and even some fish species from various ecosystems. Environmentally relevant PCB and DDE metabolites are also potential endocrine disrupters in exposed species. In Arctic species, PCB and DDE biotransformation in leading to $MeSO_2$-PCB and –DDE and OH-PCB formation occurs in, e.g., ringed seal (*Phoca hispida*), polar bear (*Ursus maritimus*) and/or beluga whale (*Delphinaterus leucas*). The metabolite levels and congener patterns are dependent on a number of factors including PCB exposure, the metabolic capacity for metabolite formation and clearance, the relative importance of metabolic pathways, and the PCB, $MeSO_2$-PCB and OH-PCB structure. We presently discuss and summarize the state of knowledge of $MeSO_2$-PCB and –DDE and OH-PCB and other phenolic OHS metabolites in Arctic biota, and the value of these metabolites as biomarkers of the importance of metabolism in the toxicokinetics and fate of the OHS parent compounds.

Contaminants in Traditional Food in Northern Canada. L.H.M. Chan. Centre for Indigenous Peoples Nutrition and Environment (CINE), Macdonald Campus of McGill University, Ste. Anne de Bellevue, QC.

The presence of environmental contaminants such as heavy metals and organochlorines in the Arctic ecosystem is posing a potential health hazard for people consuming traditional foods in the northern communities. Monitoring the concentrations of these pollutants in the traditional food system has become a priority for risk management purposes. Between 1995 and 1999, we have collected 546 samples from 29 communities in different regions in Northern and Arctic Canada and analyzed the concentrations of heavy metals and/or organochlorines. A significant percentage of the foods had contaminant levels exceeding the guidelines used by Health Canada for market food consumed by the "southern" populations. We will discuss the approaches of using this data set to conduct risk assessment of dietary exposure of environmental contaminants. (Supported by the Northern Contaminants Program of Indian and Northern Affairs Canada.)

Organochlorine Pesticide and PCB Concentrations in Amphipods (*Gammarus lacustris*) Along an Elevation Gradient in Mountain Lakes of Banff National Park, Canada. J.M. Blais[1,2], F. Wilhelm[3], K.A. Kidd[4], D.C.G. Muir[5], D.B. Donald[6] and D.W. Schindler[1]. [1]Department of Biological Sciences, University of Alberta, Edmonton, AB; [2]Department of Biology, University of Ottawa, Ottawa, ON; [3]Department of Zoology, University of Otago, Dunedin, New Zealand; [4]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; [5]Environment Canada, National Water Research Institute, Burlington, ON; [6]Environment Canada, Regina, SK.

Populations of the amphipod *Gammarus lacustris* were examined for their concentrations of organochlorine pesticides and polychlorinated biphenyls (PCBs) from seven lakes spanning a 1300 m elevation gradient in Alberta, Canada. Several of the more volatile compounds (MVC) increased in concentration at higher altitudes, whereas less volatile compounds (LVC) did not show this pattern. These same MVC compounds have been previously shown to increase at high latitudes as a result of their preferential long-range transport and deposition in cold climates. We also show that alpine populations of *G. lacustris* have longer life cycles, slower growth rates, and store more lipids than their prairie and montane counterparts, all of which have been shown to favor enhanced bioaccumulation of these chemicals in animals. This study confirms that a pattern of chemical enrichment is observed at high altitudes, with higher concentrations of MVCs in alpine *Gammarus* relative to lower-altitude populations.

Use of White Suckers (*Catostomus commersoni*), Northern Pike (*Esox lucius*) and Lake Whitefish (*Coregonus clupeaformis*) to Determine the Bio-Availability of Metals and Trace Elements Discharged from Uranium and Gold Mining Developments in the North. J.F. Klaverkamp, C.L. Baron, B.W. Fallis and C.R. Ranson. Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

Guidance statements for implementation of the Environmental Effects Monitoring (EEM) Program for metal mining in Canada include determination of the magnitude and extent of contaminant discharges into receiving waters and the bio-availability of contaminants to fishes. Analyses of sediments from lakes receiving uranium mining effluents in northern Saskatchewan reveal surficial enrichment factors ranging from about 5 to 720 for As, Co, Cu, Mo, Ni, and Se.

Analyses of livers and kidneys of suckers, pike and whitefish from these lakes indicate contaminant concentrations up to 43 times higher than concentrations observed in fish tissues from reference lakes. Sediments from sites in Yellowknife Bay (Great Slave Lake) contaminated by gold mining and milling operations contain As, Cu, Ni, and Zn concentrations about 3 to 270 times higher than concentrations measured in a reference site in the Bay. With some exceptions, livers and kidneys of northern pike and lake whitefish captured at the contaminated sites, contain significantly higher concentrations of As, Cu and Zn. Metallothionein induction and element-to-element interactions in livers and kidneys of fishes from aquatic ecosystems impacted by these uranium and gold mining and milling operations will also be presented.

Hydrocarbon Studies in the Lake Athabasca and the Athabasca River Ecosystem: PAH Concentrations, Compositions, Time Trends, and Sources. M.S. Evans[1], W.L. Lockhart[2], B. Billeck[2], G. Stern[2], L. Noton[3], J. P. Bechtold[4], A. Cummins[5], N. Easterbrook[6], B. Hunter[7], B. Kemper[8], B. Ross[2] and M. Yunker[9]. [1]Environment Canada, National Water Research Institute, Saskatoon, SK; [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; [3]Alberta Environment, Edmonton, AB; [4]Golder Associates, Calgary, AB; [5]Suncor Energy Inc., Fort McMurray, AB; [6]Shell Canada Ltd., Fort McMurray, AB; [7]Syncrude Canada Ltd.; [8]Kemper & Associates, BC; [9]Brentwood, BC.

It recently has become economically feasible to extract hydrocarbons from the immense oil sands reserves that lie on either side of the lower Athabasca River, north of Fort McMurray, AB. Through research (PERD) and monitoring (RAMP) programs, we are investigating the potential impact of these operations on hydrocarbon (mainly PAHs) concentrations, composition, and time trends in tributaries, the Athabasca River and Delta, and Lake Athabasca. Data are compared with earlier studies in Great Slave Lake and the Mackenzie River Delta.

Hydrocarbon concentrations tend to be intermediate between the lower values observed in remote Arctic Ocean locations and lakes on the Precambrian Shield but not as high as in the Mackenzie River delta. Compounds of petrogenic origin dominate but combustion sources also are important. Highest PAH concentrations are associated with fine-grained sediments in depositional areas. There is limited evidence of temporal trends of PAH increase in sediment cores collected in downstream lakes. Some low molecular-weight PAHs exceed interim sediment quality guidelines and some bioassay studies have shown some evidence of toxicity. The RAMP monitoring program will continue to assess the potential impacts of the oil sands industry of this important aquatic ecosystem.

Zoobenthic Succession of Constructed Wetlands in the Fort McMurray, Alberta Oil Sands Region. C. Leonhardt and J.J.H. Ciborowski. Department of Biological Sciences, University of Windsor, Windsor, ON.

We evaluated the successional phenology of benthic invertebrate community development of three classes of wetlands formed in oil sands mining areas. Our goal is to characterize and ordinate communities with respect to time since reclamation, and to produce a scale by which to measure progress towards attainment of a reference state. We sampled 21 wetlands aged 1 to 28 years, using sweep nets, sediment cores, and artificial substrates and identified the animals to genus. Six low-conductivity reference wetlands (<650 μS/cm; formed following construction activity), and 8 intermediate-salinity wetlands (1250 to 2400 μS/cm; built on reclaimed mining sands) contained natural surface waters. Seven higher-saline wetlands (1250 to 4600 μS/cm) sampled were built on waste materials (mining sands and/or consolidated tailings). These had or are receiving oil sands process-affected water (OSPW) and natural surface water.

Zoobenthic family richness increased with wetland age, but was negatively related to salinity and naphthenic acid content (indicators of similarity to OSPW or degree of bioremediation). Sediment organic matter content was a better predictor of benthic richness and density than age. Young wetlands (<7 years) of each class were dominated by different types of midges (Orthocladiinae and Tanypodinae for OSPW-affected wetlands, Chironomini and Tanytarsini/Tanypodinae for high conductivity wetlands, and Tanytarsini and Orthocladiinae for low conductivity wetlands). Zoobenthic community similarity converged among older wetlands (>13 years), all classes becoming increasingly characterized by oligochaetes, Cladocera, Odonata and Ephemeroptera.

The Effectiveness of Oil Bioremediation Processes in Marine Sediments under Sub-Antarctic Conditions. É. Pelletier[1], D. Delille[2] and B. Delille[3]. [1]Institut des Sciences de la Mer de Rimouski (ISMER), Université du Québec à Rimouski, Rimouski, Qc; [2]Laboratoire Arago, Université Paris VI, France; [3]Institut de Physique, Université de Liège, Belgique.

The effectiveness of crude oil bioremediation using various fertilisation treatments was evaluated under environmental conditions encountered in sub-Antarctic environments. Experimental plots were settled in a remote sandy beach of the main island of the Kerguelen Archipelago (49°19'S, 69°42.5'E), a pristine environment where the hydrocarbon degrading bacteria (HDB) community has not been stimulated by previous accidental spills. Chemical, microbial and toxicological parameters clearly demonstrated the effectiveness of various fertilisers (Inipol® and fish flours) applied to an Arabian light crude oil spilled in the intertidal zone at the mid-tide mark.

The toxicity of oiled sands, as evaluated by Microtox® solid phase bioassay, was significantly reduced by a successful bioremediation in the first 200 days of the process, but it started to increase again when most alkanes and aromatics appeared to be degraded. A linear correlation between the alkanes degradation rate and the growth rate of HDB was present up to day 90 when the bacterial community was at its maximum of growth and degradation activity. Interstitial waters collected bellow the oil slicks during the remediation process showed no toxicity and were even stimulating *Vibrio fischeri*. Finally, the low temperature of seawater (3 to 4°C) during the sub-Antarctic winter had apparently no effect on the HDB community as most of the bioremediation process occurred during the winter. Although assessing *in situ* bioremediation is a very complex task plagued by methodological limitations, heterogeneity of the medium and uncontrolled environmental events, our work integrated chemical, microbial and toxicological parameters and clearly demonstrated the effectiveness of various fertilisers applied to crude oil in a pristine environment under severe weather conditions.

Endocrine Disruptors

Session Co-chairs: K.A. Kidd and V.P. Palace

An ELISA for Atlantic Salmon (*Salmo salar*) Vg and its use in Measuring the Response of Salmon Smolts to 17β-estradiol and 4-Nonylphenol Treatments. J.P. Sherry[1], C. Tinson[1], K. Haya[2], L.E. Burridge[2], W.L. Fairchild[3] and S.B. Brown[1]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Department of Fisheries and Oceans, Biological Station, St. Andrews, NB; [3]Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB.

Fairchild *et al.* (1999, Environ. Health Perspect. 107: 349-357) used an epidemiological approach to associate declines in returning populations of Atlantic salmon (*Salmo salar*) in several Eastern Canada rivers to applications of Matacil® 1.8D in forest spray operations to control Spruce budworm. A plausible mechanistic link was suggested to 4-nonylphenol, which is the diluent in commercial formulations of the insecticide. Laboratory experiments with salmon smolts demonstrated that 4-NP could impair subsequent growth and survival in sea water. Since 4-NP is a known environmental estrogen and toxicant, we wish to test whether its action in smolts is mediated by an estrogenic mechanism, or whether some other mode of action is involved. The egg yolk protein vitellogenin (Vg) was selected as a response signal for the estrogenic pathway since it can be induced by various estrogenic chemicals including 4-NP. An indirect competitive binding enzyme-linked-immunosorbent-assay, which was based on commercially available antibodies, was developed and optimized. The ELISA was used to measure Vg in the plasma of salmon smolts that had been exposed to 17β-estradiol and 4-nonylphenol under a flow through regime. The ELISA data was confirmed by PAGE and Western Blotting techniques. The preliminary experimental data show that both test chemicals induced Vg in a dose dependent manner in the exposed smolts.

A Summary of the Reproductive-Endocrine Effects of a New Zealand Pulp Mill Effluent. M.R. van den Heuvel[1], R.J. Ellis[1], E. Bandelj[1,2], L.H. McCarthy[2] and T.R. Stuthridge[1]. [1]New Zealand Forest Research Institute, Rotorua, New Zealand, [2]Ryerson University, Toronto, ON.

Numerous studies report reproductive-endocrine responses in fishes exposed to pulp and paper mill effluents. A three-year, multi-species study has been carried out to determine the potential of a New Zealand mixed bleached-kraft/thermomechanical pulp and paper mill effluent to elicit reproductive effects similar to those observed elsewhere. Experiments were carried out with final (secondary treated) effluent at concentrations relevant to this particular receiving environment.

Studies with rainbow trout vitellogenin and estrogen receptor expression have demonstrated that this effluent is not likely to act in an estrogenic manner. Large-scale mesocosm studies with sexually mature rainbow trout have demonstrated that reduced gonad size can be induced after an eight-month exposure that was initiated well prior to the initiation of sexual maturation. Gonad size differences were correlated with levels of circulating sex steroid hormones prior to sexual maturation. Strength of correlations decreased as gonadal growth advanced. Trout that were exposed starting two months prior to spawning showed no gonad growth or steroid effects. Experiments with mosquitofish demonstrated that this effluent has the ability to induce the development of male secondary sexual characteristics in females. Subsequent investigations have shown that effluent extracts contain compounds that bind to goldfish androgen receptors *in vitro*. Results to date indicate that this particular pulp mill effluent is eliciting effects through a mechanism that peripherally appears to be androgenic in nature.

Pulp Mill Effluent Affects Growth and Secondary Sex Characteristics of Fathead Minnows. J.L. Parrott[1], C.S. Wood[2], P. Boutot[2], B.R. Blunt[1], G.G. Fodor[1], M.A. Baker[1] and S. Dunn[3]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Nexfor Technology Centre, Pointe Claire, QC; [3]Fraser Paper Inc., Edmundston, NB.

During Environmental Effects Monitoring studies, wild fish collected downstream of Nexfor Edmundston bleached sulphite mill (BSM) had reduced gonad size and fecundity. To assess BSM final effluent, long-term growth and reproduction tests were carried out in a flow-through bioassay trailer at the mill secondary treatment lagoons. Fertilized fathead minnow eggs were exposed to 0, 1, 3.2, 10, 32, 48 and 100% final effluent (or ethinylestradiol positive control).

The effluent caused a significant increase in the growth of fish (at 10 to 48%) at 30, 60 and 125 d post-hatch. Changes in external sex characteristics, such as ovipositor index, were seen at 60 d post-hatch in 32 and 48% effluent. At 125 d, some male fish had ovipositors in effluent concentrations as low as 3%. Higher effluent concentrations (32 to 100%) resulted in a majority of fish that looked externally like females. Fish exposed to 1% effluent were the only ones to produce eggs in the study, despite significantly increased condition factors in males and females from 10 to 48% effluent. Eggs from the fish exposed to 1% effluent had >90% fertilization and hatching success. Concentrations of final effluent in the Saint John River range from less than 1 to 15%, depending on the season and river flow.

Potential Endocrine Disruption in Freshwater Systems near Agricultural Areas on Prince Edward Island. R. Mroz[1], W.R. Ernst[1], L. Rutherford[1], K. Kennedy[1], J.P. Sherry[2], P. Falletta[3] and E. Bentley[4]. [1]Environment Canada, Atlantic Region, Dartmouth, NS; [2]Environment Canada, National Water Research Institute, Burlington, ON; [3]Environment Canada, Wastewater Technology Centre, Burlington, ON; [4]Bedeque Bay Environmental Management Assoc., Summerside, PE.

A field study was undertaken on Prince Edward Island in 1999 and 2000 to evaluate the potential of agricultural chemicals to cause endocrine disruption in freshwater ecosystems. Study sites were located in potato growing areas of Prince Edward Island due to the intensive use of pesticides on that crop compared to others. This paper presents results from the analysis of caged fish and wild fish for plasma vitellogenin concentrations, brain cholinesterase levels, liver MFO concentrations and a bile yeast estrogen-screening assay. Rainbow trout (*Oncorhynchus mykiss*) were caged in various rivers to coincide with the most intense pesticide applications (mid-July to mid-Sept) and at the end of the exposure period, were sacrificed to collect blood and tissue samples. A pesticide and land-use survey was used to verify exposure. Positive controls were established by injecting additional fish with 17β estradiol and placing them in a cage adjacent to the non-injected fish. Water samples were analyzed for pesticide and nonylphenol concentrations. Quantitative electrofishing was conducted to determine whether brook trout populations were affected by pesticide use. Tissue and blood samples of the wild brook trout were collected during the electrofishing and subjected to the same assays as the caged fish. All data is pending final QA/QC review and will be available for presentation at ATW.

A Whole-Lake Experiment to Examine the Effects of a Synthetic Estrogen on Fish Populations. K.A. Kidd[1], V.P. Palace[1], P. Blanchfield[1], K. Mills[1], R.E. Evans[1], M.E. McMaster[2], S.B. Brown[2], G.J. Van Der Kraak[3], D. Lattier[4] and J. Lazorchak[4]. [1]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; [2]Environment Canada, National Water Research Institute, Burlington, ON; [3]Department of Zoology, University of Guelph, Guelph, ON; [4]US EPA, Cincinnati, OH.

There is increasing evidence that natural and synthetic estrogens in sewage treatment plant effluents are impacting endocrine function and gonadal development in wild fish. It is not known whether the effects observed in individuals, such as vitellogenin production and reduced gonad size, are indicative of problems with a population's sustainability. We are currently in the third year of a whole-lake manipulation at the Experimental Lakes Area, northwestern Ontario to examine the link between individual- and population-level effects in fish exposed to environmentally-relevant concentrations of the synthetic estrogen ethynylestradiol (EE2). Two years of baseline studies were completed in 1999 and 2000 on the lake trout, white sucker, fathead minnow and pearl dace populations in several lakes. In 2001, EE2 is being added continuously one lake over the open-water season. Sex steroids, thyroid hormones, gonadotropin, vitellogenin and vitellogenin mRNA, gonadal development and reproductive behaviour are being examined in the effected populations and contrasted with baseline and reference lake data. In addition, we are monitoring population-level effects such as age to maturity, growth rates, and abundance. Results will contribute to our understanding of the risks that hormone mimics pose to wild populations, and of the relationship between responses across levels of biological organization.

Freshwater Fish Exposed to Environmental Estrogens have Altered Lipid Soluble Vitamin Status. V.P. Palace[1,2], K.A. Kidd[1,2], K. Wautier[1], R.E. Evans[1], T.A. Dick[2], J. Werner[2] and C.L. Baron[1]. [1]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; [2]Department of Zoology, University of Manitoba, Winnipeg, MB.

Altered metabolism of the lipid soluble vitamins A and E has been reported for several classes of organic contaminants in a wide range of vertebrate species. Furthermore, it has been postulated that enhanced vitamin clearance rates in contaminant-exposed organisms may contribute to their reproductive impairment. With the recent emphasis on endocrine active environmental contaminants and the reproductive capacity of exposed organisms, questions regarding the metabolism of lipid soluble vitamins have also been raised with regard to this class of contaminants. The questions are especially relevant where estrogenic contaminants are concerned given evidence from mammals that shows levels of vitamin A and E can be significantly affected by exposure to estrogenic compounds. Results from a series of experiments will be presented demonstrating that vitamin levels are affected in freshwater fish exposed to environmentally relevant concentrations of the environmental estrogen, ethynylestradiol. Implications for altered viability of offspring from vitamin –compromised adult females will also be discussed.

Effects of Ethynylestradiol on Early Development of Amphibians in a Boreal Lake. B.J. Park[1] and K.A. Kidd[2]. [1]Department of Zoology, University of Manitoba, Winnipeg, MB; [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

Amphibian populations are exposed to substances that can impact development through modulation of the endocrine system. In this study the effects of a known hormone mimic on embryonic and early larval amphibians were examined. A study lake was dosed with 17α-ethynylestradiol (EE2) at a mean concentration of 6 ng/L, in June to September 2001 (Experimental Lakes Area, NW Ontario, Canada). Egg masses of mink frogs and green frogs (*Rana septentrionalis* and *R. clamitans*) were collected from the wild. Subsamples of the egg masses were maintained in enclosures on the EE2-treated lake and two reference lakes from July to September 2001 (embryonic stage to tadpole hindlimb formation). Hatch rates, survival, and growth and development rates were recorded.

Hatch rate for green frog eggs were lower on the EE2-treated lake than on reference lakes, though the effect was no statistically significant. There were no significant differences among lakes for survival and growth and development rates. No endocrine-disrupting mechanisms are known for embryonic amphibians. The role of endogenous hormones in early development (premetamorphosis) is unclear, but physiological changes at metamorphosis are under direct control of thyroid hormones. The current data suggest that low-level EE2 exposure in the wild has a toxic effect in green frog embryos and does not impact early development in either species. Ongoing research will elucidate EE2 effects on endogenous thyroid hormone levels, gonad development, and growth and development rates in later-stage tadpoles.

Toxicity and People

Session Co-chairs: W.G. Foster and M.G. Wade

Who? When? Where? What? How Long? J.H. McCormick. U.S. Environmental Protection Agency, Duluth, MN (Retired).

As scientists, we are trained to answer questions. Over time we get pretty good at this. As aquatic environmentalists, what we have to do is to ask the right questions. The following presentation is an initial set of fundamental questions that should be answered before making decisions relative to anticipated effects of various proposed, expected, or actual environmental perturbations.

Presence of Environmental Contaminants in Human Follicular Fluid, Serum and Seminal Plasma of Couples Undergoing *in vitro* Fertilization. E.V. YoungLai and W.G. Foster. Department of Obstetrics & Gynaecology, McMaster University, Hamilton, ON.

In order to explore the relationship between exposure to environmental pollutants and *in vitro* fertilization (IVF) outcome, follicular fluid was collected from the female partner and seminal plasma from the male partner of 21 couples attending an IVF program. Serum was collected from both partners. Sera and fluids were analyzed for contaminants including polychlorinated biphenyls, pesticides, Cd, and cotinine. Of the couples examined three became pregnant and there were no fertilizations in another three.

The dominant contaminants found in follicular fluid were *p,p'*-DDE, mirex, hexachloroethane, 1,2,4-trichlorobenzene, methoxychlor, heptachlor epoxide, PCB 49, PCB 138, PCB 153 and PCB 180. Seminal plasma was relatively free of pollutants with mirex being the major pesticide found in 7 samples. Sera were highly contaminated with *p,p'*-DDE, 1,2,4-trichlorobenzene, endosulfan I, PCB 99, PCB 118, PCB 138, PCB 153, PCB 170, PCB 180 and PCB 187. Cd was not detectable in 14 samples of follicular fluid whereas cotinine was not detectable in 3. No relationship was found between fertilization or pregnancy and residue levels in any reproductive fluid and serum. However, follicular fluid cotinine was higher in the patients where fertilization did not occur (462 ± 341 µg/L) compared to those who became pregnant (199 ± 104 µg/L). Although no link between exposure and adverse outcome could be demonstrated the finding of environmental contaminants in human reproductive fluids raises concern.

Thyroid Hormone Disruption by a Complex Mixture of Ubiquitous Environmental Contaminants. M.G. Wade, S. Parent, W.G. Foster, E.V. YoungLai, A. McMahon and C.L. Hughes. Health Canada, Environmental Health Directorate, Ottawa, ON.

Disturbance of normal thyroid physiology can have significant effects on the development and function of the central nervous system and development of the reproductive tract. The human population of the developed world is ubiquitously exposed to a variety of substances, such as dioxins, PCBs, chlorinated benzenes, etc., shown to disrupt thyroid homeostasis in laboratory animals. Very little is known about how these substances interact in inducing this toxicity in spite of the fact that the majority of humans have measurable quantities of all of these in their tissues albeit at levels below those shown to cause thyroid effects. A series of experiments were conducted to determine the toxicity of a complex mixture of ubiquitous, persistent contaminants to thyroid physiology. The mixture was formulated by including 16 organochlorine contaminants and two metals that are routinely detected in tissues of Canadians, to be administered at doses equivalent to estimates of acceptable or tolerable daily exposure of each component. Two sets of dose-response experiments were conducted, one examining subchronic effects where the mixture was administered daily, for 70 d, to sexually mature male rats and a second examining effects on development where the mixture was administered to pregnant female rats on gestational days 9 through 15.

The mixture had low general subchronic toxicity in males as demonstrated by lack of effects on body weight and effects on organ weights only at the highest dose tested (1,000X). In contrast, treatment with the mixture had significant effects on all endpoints related to thyroid homeostasis including circulating thyroxine (T4), triiodothyronine (T3) and thyroid-stimulating hormone (TSH) levels, serum T3 binding capacity, several endpoints of thyroid gland histomorphology and activity of hepatic thyroxine metabolizing enzymes UDP glucuronyl transferase and outer ring deiodinase (ORD). The most sensitive endpoints were circulating TSH levels and ORD activity, which were significantly altered by 1X MRL mixture treatment, whereas other measures were only altered by 100X MRL and higher doses. As both endpoints are proteins that are directly influenced by thyroid hormone action, these results suggest that the thyroid hormone signal is reduced at the tissue level by the lowest dose although circulating T4 levels, the endpoint most widely considered as indicative of thyroid status, is reduced only in the highest dose animals. The mixture causes minimal changes in thyroid physiology in pregnant rats or their offspring except that there is a significant increase in circulating thyroid hormones in the male, but not female, offspring well after the treatment has ceased. These results demonstrate that this mixture of ubiquitous contaminants is a potent disruptor of thyroid hormone status but that the consequences of developmental exposures to this mixture are not clear.

Lead and POPs in the Mushkego Cree. B.C. Wainman[1], L.J.S. Tsuji[2], E. Nieboer[3] and I.D. Martin[4]. [1]Department of Obstetrics and Gynecology, McMaster University, Hamilton, ON; [2]Department of Environmental and Resource Studies, University of Waterloo, Waterloo, ON; [3]Department of Biochemistry, McMaster University, Hamilton, ON; [4]Aquatics Analysis, Elora, ON.

The potential disruption of health because of persistent organic pollutants (POPs) and toxic metals is a serious concern for the Cree living in the Mushkegowuk Region (western James Bay). Our preliminary results for 32 males and 35 females from James Bay revealed plasma PCBs concentrations (measured as Aroclor 1260; geometric mean and range) of 495 (55 to 4,266) µg/kg lipid for the females and 559 (132 to 3,548) µg/kg lipid for the males; and for p,p-DDE of 341 (51 to 1,995) µg/kg lipid in females and 318 (87 to 2,344) µg/kg lipid in males. These levels are comparable (for PCBs) or greater by a factor of 2 or more (DDE) than those reported for First Nations people living in Canada's arctic regions who, like the Cree, do not consume marine mammals. Compared to a control group in Hamilton ON (n=50), the Mushkego Cree have significantly higher blood Pb levels (geometric means and range): 60 (15 to 178) µg/L (males, n=50) and 36 (9 to 137) µg/L (females, n=48) compared to 21 (9 to 68) µg/L (males, n=25) and 25 (12 to 110) µg/L (females, n=19) in Hamilton. The probable route of contaminant uptake is through ingestion of fish (for the POPs) and waterfowl (for the lead). These contaminant levels are of particular concern because the Cree are linked to their environment by the consumption of traditional foods and cannot avoid these foods for economic and cultural reasons.

Unweaving the Web of Life: Persistent Toxic Substances and the Potential Risks to First Nations Women and Children in the Great Lakes Region. S. Allen[1] and R. Whitlow[2]. [1]North Shore Tribal Council, Cutler, ON; [2]Chiefs of Ontario, Brantford, ON.

The Eating Patterns Survey (EPS) was one research component of the E.A.G.L.E. (Effects on Aboriginals from the Great Lakes) Project. The primary objective of the E.A.G.L.E. EPS was to quantify the exposure of First Nations peoples in the Great Lakes basin to persistent toxic substances (PTSs) through the consumption of freshwater fish and wild game, and to use this information to examine the potential risks to health. The EPS was administered via a questionnaire designed to provide information on the amount and frequency of consumption of different species of freshwater fish and wild game, the different proportions of each consumed, and pertinent demographic information. The final results represent those of 30 First Nations and a total of 1,658 individuals of which 83.4% consumed freshwater fish in the last year. In terms of the amount of all fish consumed, the five most frequently consumed species of fish basin-wide were: pickerel, whitefish, lake trout, perch and northern pike. The complete list of freshwater fish consumed basin-wide included 19 different species and a category for others.

The key segments of any population, including First Nations, who may be at risk of adverse health effects due to the consumption of freshwater fish, are children; women in their reproductive years; "high" consumers of freshwater fish; and individuals with sensitivities or confounding health conditions. EAGLE was unable to identify the latter individuals because this information was not included in the EPS questionnaire. However, we are able to identify the members of the first three high-risk groups from the EPS data. From the data, it appears that 13% of adults are eating freshwater fish on a weekly basis throughout the year and the top 5% of consumers are eating more than 2 meals per week on a yearly basis, ranging from 108 to 394 meals per year. Approximately 10% of women in their reproductive years are eating freshwater fish on a weekly basis and the top 5% are eating from 64 to 191 freshwater-fish meals in one year. The findings for children indicate that approximately 10% are consuming freshwater fish on a weekly basis. The top 5% of children consuming freshwater fish as surveyed here are consuming 71 to 212 freshwater-fish meals in one year.

The findings of the E.A.G.L.E. EPS provide clear evidence that Great Lakes First Nations consume large quantities of fish, significantly more than the general Canadian population. This consumption represents slightly more than that of the average non-First Nation "high consumer" (i.e., anglers). Given the results for consumption of freshwater fish, First Nations peoples in the Great Lakes basin are at greater risk for exposure to persistent toxic substances and this in turn increases the likelihood of associated health effects. The results for the "at-risk" population (high consumers, women in their reproductive years, and children) from this study are significant and indicate that further analysis of this data and further research is required to address the concerns for "at-risk" members of the First Nations populace in the Great Lakes region.

EDCs, First Nations and the Role of Toxicology. M.-A.S. Phare[1], J.M. McKernan[2], R.D. Breu[1] and P. Larcombe[1]. [1]Centre for Indigenous Environmental Resources; [2]TetrES Consultants Inc., Winnipeg, MB.

Endocrine Disrupting Compounds (EDCs) are a large class of hormonally active industrial chemicals and pesticides that can affect people by interfering with normal functioning of the human and wildlife endocrine (especially reproductive) systems. Releases of many EDCs to the environment are not regulated, and the thresholds for morbidity have not been established for many other EDCs. As well, the long-term effects of EDC exposures are unknown or uncertain, especially for low-level chronic exposures. Certain human-health effects are of particular concern to First Nations (e.g., reproductive failure, disruption or dysfunction).

Many First Nations may be particularly vulnerable to chronic EDC exposures because of: [1] isolation, (a) First Nations have higher risks of exposure to effluents from pulp mills, mines, smelters, etc., that are located in rural and northern areas where First Nation communities tend also to be located, (b) First Nations are usually dependent on sustenance from land and water bases, and (c) First Nations have limited or no access to state-of-the-art scientific knowledge and/or on-site toxics-monitoring capability, [2] Potential exposure to EDCs of all kinds in relation to diet, cultural activities, and geography, [3] Legal and political status (a) First Nations are generally excluded from meaningful participation in planning and decision-making regarding activities that may impact the environment where they live, (b) First Nations are dependent upon the government to fulfill its fiduciary relationship regarding environmental protection of First Nation lands and territories, and (c) First Nations are largely dependent on others for scientific knowledge and technology, [4] Physiology; e.g., a possible but unknown predisposition or susceptibility to diseases.

As a group of marginalized people, with possible particular exposure and susceptibility to EDCs, they have nevertheless been largely ignored in the current round of EDC studies. With few exceptions, toxicology is not now focused on the question of First Nations exposures to EDCs, and the significance of such exposures. Insufficient prioritization of attention from the scientific and political communities on this issue, and insufficient assistance for First Nations to fully address the issue, means that mechanisms that may affect survival of First Nations gene pools (e.g., EDC-constrained fecundity or reproductive success) are allowed to persist without rigorous scrutiny. Some groups in society would argue that failure to engage in such scrutiny, and in the empowerment of First Nations to assist in protecting themselves from EDC exposures, means that the discipline of toxicology is failing to be socially responsible. These groups might argue that this is tacit tolerance of "environmental racism". As well, federal and provincial law, treaties, Constitutional recognition and affirmation of Aboriginal rights, and the fiduciary obligation of both levels of government mandate that study of First Nation's susceptibilities to EDC exposures be treated with the seriousness it deserves.

This is a pressing priority that remains unattended in Canada. Application of the common-sense precautionary principle would suggest that First Nations should be a priority subject of study, given the foregoing. Toxicology can increase its relevance and its social responsibility by focusing, among other things, on this issue.

Alylphenols

Session Co-chairs: M.R. Servos and E.M. Mihaich

Nonylphenol Ethoxylate Surfactants - History, Use, and Safety. E.M. Mihaich[1], C.G. Naylor[2] and C.A. Staples[3]. [1]Rhodia Inc., Raleigh, NC; [2]Huntsman Chemical Corp., Austin, TX; [3]Assessment Technologies, Inc., Fairfax, VA.

Nonylphenol ethoxylate (NPE) surfactants have been safely used for more than 50 years, in part due to inherent properties that make them a high-performance and cost-effective choice in a wide range of uses. NPEs are found in a broad range of applications such as household, industrial, and institutional cleaners, as processing aids in the manufacture of textiles and paper, as adjuvants in agricultural products, in the manufacture of latex polymers in water based paints, and in emulsion polymerization technology. Nonylphenol and nonylphenol ethoxylates are biodegradable in water and sediment and are easily degraded in biological treatment systems. A mounting number of initiatives around the world addressing alkylphenol and alkylphenol ethoxylates have focused attention on their use and disposal. The main areas on which scientific assessments of NPE/NP focus are the aquatic and terrestrial environments. Most recently, the specter of alkylphenols as potential disrupters of the endocrine systems of man and animals is being raised with increasing frequency and has spawned considerable scientific research. NPEs and their breakdown products are part of perhaps the most "studied" surfactant family available, the subject of hundreds of toxicity, environmental fate and effects studies. The knowledge base of these products' environmental and human health properties is large and more extensive than exists for other, newer surfactants that serve similar purposes.

A Preliminary Assessment of Ecological Risks from Nonylphenol in Municipal Sewage Sludge Following Wastewater Treatment. C.A. Staples[1], E.M. Mihaich[2], C.G. Naylor[3] and B.E. Huntsman[4]. [1]Assessment Technologies, Inc., Fairfax, VA; [2]Rhodia, Inc., Raleigh, NC; [3]Huntsman Chemical Corp., Austin, TX; [4]Terran Corp., Beavercreek, OH.

NPE surfactants are biodegraded in biological wastewater treatment plants. Final effluents discharging to surface waters typically contain measurable amounts of nonylphenol mono- and di-ethoxylates and nonylphenol mono- and di- ether carboxylates, but only trace levels of nonylphenol. Potential aquatic risks from these biodegradation intermediates were recently examined in a comprehensive assessment conducted according to *Canadian Environmental Protection Act* requirements. However, most nonylphenol formed during wastewater treatment is associated with sludge that is disposed in landfills, incinerated or used as a soil amendment. Detailed assessment of potential risks from sludge-amended soil containing nonylphenol has not been conducted. A preliminary ecological risk assessment is presented here that examines potential risks from nonylphenol in sludge-amended soil. The main pathways assessed were direct exposure of soil to earthworms, insects, and plants, and ingestion of soil, invertebrates, and plants by small mammals. Also, the potential for migration to groundwater was assessed. Preliminary results show that potential risks to the terrestrial environment from sewage sludge applications containing nonylphenol are negligible and that groundwater is not being impacted. The results of this preliminary ecological risk assessment are being supplemented by further studies to refine uptake from sludge-amended soil by a variety of plants and to define sludge concentrations at a number of sites.

Occurrence, Fate and Release of Alkylphenol Polyethoxylates in Municipal Effluents and Industrial Effluents in Canada. D.T. Bennie[1], M.R. Servos[1], B.K. Burnison[1], P. Seto[2] and A. Schnell[3]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Environment Canada, Wastewater Technology Centre, Burlington, ON; [3]Hydromantis, Inc., Cambridge, ON.

Between 1997 and 2000, Environment Canada conducted a nation-wide survey of more than 20 municipal wastewater treatment plants, representing a wide range of treatment processes, conditions of operation, and influent characteristics, to investigate the occurrence and release of selected endocrine disrupting substances in effluents and sludges. In the final year of the study, six pulp and paper mills and three textile mills in eastern Canada were also surveyed. With one exception, this involved short-term, i.e. one to three days, sampling and monitoring programs to determine the concentrations and loadings of endocrine disrupting substances in raw sewage, treated effluent and sludge. Samples were collected and analyzed for alkylphenolic substances, selected natural and synthetic estrogens (e.g., 17β-estradiol, estrone and ethenyl estradiol) and other substances found on the *Canadian Environmental Protection Act* (CEPA) Second Priority Substances List (PSL 2). This presentation will focus on the measurements of alkylphenol polyethoxylates (NP(3-17)EO) and their degradation metabolites, i.e. alkylphenols (NP and 4-t-

OP), alkylphenol ethoxylates (NP1EO and NP2EO) and alkylphenoxyacetic acids (NP1EC, NP2EC, OP1EC and OP2EC).

The occurrence and environmental release of significant concentrations of alkylphenolics was confirmed by the nationwide survey. In general, it appeared that WWTP plants utilizing biological treatment processes were able to reduce levels more effectively and plant loading was significant factor in achieving low discharge levels. As expected, 4-NP was found to accumulate on anaerobically digested sludge and aerobically digested sludge samples contained lower amounts of alkylphenolic substances. The secondary treated effluent from the six pulp and paper mills contained negligible amounts of alkylphenolics while untreated effluents discharged from the three textile mills contained moderate but quite variable concentrations of these substances.

The Effects of Alkylphenols on Growth of Atlantic Salmon Smolts. S.B. Brown[1], K. Haya[2], L.E. Burridge[2], D. Bennie[1], J.T. Arsenault[3], R.E. Evans[4], B.K. Burnison[1], J.P. Sherry[1], J.G. Eales[5], D.L. MacLatchy[6] and W.L. Fairchild[3]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Department of Fisheries and Oceans, Biological Station, St. Andrew's, NB; [3]Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB; [4]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; [5]Department of Zoology, University of Manitoba, Winnipeg, MB; [6]Department of Biology, University of New Brunswick, St. John's, NB.

We investigated the hypothesis that alkylphenols, like *p*-nonylphenol (4-NP) and nonylphenol-1-carboxylate (NP1EC), may perturb parr-smolt transformation (PST) and subsequent sea water growth in Atlantic salmon (*Salmo salar*). Fish were exposed to environmentally relevant, doses of water-borne 4-NP, NP1EC and 17*ß*-estradiol (E2) during the latter stages of PST. The capability of smolts to withstand sea water and their subsequent growth were evaluated. We also assessed the status of various osmoregulatory, hormonal and histological parameters. There were no treatment related increases in mortality during a sea water challenge immediately after exposure. However, subsequent growth in sea water was impaired in 30 to 40% of fish from the various treatment groups. In some treatment groups there were changes in biochemical indices of growth, thyroidal status, plasma steroids, vitellogenin, and histology but not osmoregulatory parameters. These findings suggest that alkylphenolic and steroidogenic activity stemming from current effluent sources (e.g., domestic sewage, agricultural wastes or phytoestrogens from pulp mills) may influence seawater growth of salmon smolts.

Environmental Risk Assessment of Nonylphenol and its Ethoxylates in Canada. M.R. Servos[1], R.J. Maguire[1], D.T. Bennie[1], B.-H. Lee[1], B.K. Burnison[1], R. McInnis[1], D. Hay[2], P. Seto[2], A. Schnell[2], N. Davidson[3] P. Cureton[3] and T. Rawn[3]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Environment Canada, Wastewater Technology Centre, Burlington, ON; [3]Environment Canada, Commercial Chemicals Evaluations Branch, Hull, QC.

In June 2001, "Nonylphenol and Its Ethoxylates" (NP/NPEs) were declared "toxic" under the *Canadian Environmental Protection Act* based on an assessment conducted as part of the Priority Substances Program (PSL-2). This designation means that this group of compounds will now enter a risk management process. The assessment included background reviews and analysis of the exposure, hazard and risk for the Canadian environment. The risk characterization included a comparison of the environmental concentrations to the levels expected to cause a variety of environmental effects, including effects on endocrine function. This analysis was conducted for NP, nonylphenol ethoxylates (NP1EO, NP2EO and NP3-17EO), nonylphenol ethoxycarboxylates (NP1EC, NP2EC) and for the combined effects. The analysis was applied to measured and predicted environmental concentrations associated with textile mill, pulp mill and municipal effluents, as well as surface waters and sediments. The use of sewage sludge as an amendment to agricultural soils was also examined. Using traditional affects endpoints, there were several sites in Canada were NP/NPE concentration might represent a potential risk to the environment. However, these sites are a small percentage of sites examined, and are typically associated directly with poorly treated effluents. Consideration of other endpoints such as effects on endocrine function may alter the interpretation because of differences in relative potencies of the various components. These effects were considered in the assessment as part of a weight of evidence approach.

Environmental Management Program for Biodegradable Nonylphenol Ethoxylate Surfactants. C.G. Naylor[1], E.M. Mihaich[2], M. Bush[3]. [1]Huntsman Chemical Corp., Austin, TX; [2]Rhodia Inc., Raleigh, NC; [3]GE Specialty Chemicals, Inc., Morgantown, WV.

In the recent draft of the *Canadian Environmental Protection Act* assessment of nonylphenol ethoxylates (NPE), guideline concentrations for nonylphenol (NP) in the environment are proposed. In response to this assessment and forthcoming water quality criteria on NP in the United States, the Alkylphenols and Ethoxylates Research Council initiated the Environmental Management Program (EMP). The program is designed to support the continued use of nonylphenol-based products by promoting good product stewardship and wastewater treatment practices. This program is structured around the following four components: [1] developing practical means for formulators and users to comply with these water quality guidelines using widely accepted product stewardship and standard wastewater treatment practices, [2] development of voluntary water quality guidelines for NPE and their transitory degradation intermediates, not just NP, [3] monitoring of selected waterways in conjunction with government agencies to help determine whether NP water quality guidelines are being met, and [4] providing technical expertise to formulators and end-users of NPE to determine appropriate control methods. While this program focuses on NPE and its degradation intermediates, the approaches and technologies used are applicable for most organic compounds and should be an integral component of best management practices.

Risk Management of Nonylphenol and its Ethoxylates Under the Canadian Environmental Protection Act, 1999. L. Desforges. Environment Canada, Environmental Protection Branch, Montréal, QC.

On June 23rd, the Ministers of the Environment and of Health published in the *Canada Gazette* a notice of their intention to recommend to the Governor in Council that Nonylphenol and its ethoxylates ("NPEs") be added to the List of Toxic Substances of the *Canadian Environmental Protection Act, 1999* (CEPA-1999). From that moment, CEPA-1999 requires the Minister of the Environment to propose a regulation or other instrument respecting prevention and control action in relation to NPEs. An additional 18 months is provided to finalize the regulations or instrument. The risk management of NPEs will primarily focus on their use and/or release in the textile industry, in the pulp and paper industry and in municipal wastewater effluents. The use of NPEs in various commercial, industrial, and institutional products will also be examined. Risk-management objectives will take into consideration the *Canadian Environmental Quality Guidelines*, the Best Available Technologies Economically Achievable ("BATEA") and the recommendations of the NPE risk assessment report. All the available risk management instruments will be assessed to determine which is best suited to meet the risk management objectives. The available risk management instruments include: regulations, pollution prevention planning, codes of practice, guidelines, etc. Consultations with all interested parties will take place during the development of the risk management instrument.

Mining Environmental Effects Monitoring

Session Co-chairs: W.R. Parker and D. Ramsey

Application of the Sediment Quality Triad Downstream of a Uranium Mine and Mill Discharge. A. Rosaasen. COGEMA Resources Inc., Saskatoon, SK.

Within MMEEM, the Sediment Quality Triad (SQT) will be one of the optional tools to be employed when an effect has been determined. The gradient design in the SQT approach can be of utility in the determination of the magnitude and spatial extent of effects, and in the subsequent development of hypotheses of possible causal relationships. After twenty years of operation, the SQT approach was applied downstream of a uranium mine and mill effluent discharge in Northern Saskatchewan. Of the three components of the SQT, sediment toxicity testing illustrated counter-intuitive results, with reference and far-field locations generally illustrating greater toxicity than near field locations. The benthic invertebrate community survey indicated significant effects were confined to the near-field. An assessment of the correlations between benthic invertebrate community structure and sediment quality suggested several potential causal relationships.

Clean-up Success and Environmental Effects at the Heath Steele Mine, New Brunswick. P.M. McKee[1] and R. Prairie[2]. [1]Beak International Inc., Brampton, ON; [2]Noranda Technology Centre, Pointe-Claire, QC.

Heath Steele operated a mine/mill complex in north-central New Brunswick between 1955 and 1999, and produced Zn, Pb, Cu and Ag concentrates. Waste rock and tailings at the site are acid generating. Heath Steele will continue to collect and treat runoff and seepages into the future to mitigate the impact of acid mine drainage (AMD). The mine lies within the Tomogonops River headwaters, draining into the Northwest Miramichi River, an important Atlantic salmon river.

Metal loadings to the environment have been dominated by Zn, with Cu present as a secondary aquatic toxicant. Over the course of Heath Steele's history, loadings of Zn to the watershed have diminished from >100 tonnes/year in the 1960s to ~30 tonnes/year after 1971, and <10 tonnes/year by the mid-1990s. Since closure, Zn loadings have dropped to <5 tonnes/year, and should diminish further as site restoration work progresses. This dramatic reduction in metal loadings was achieved by interception and treatment of AMD by Noranda Inc. and predecessor owners.

Environmental Effects Monitoring (EEM) has been carried out in the Northwest Miramichi and

Tomogonops rivers beginning in the 1960s, and progressing through to present. This long-term EEM has incorporated measurement of water quality, acute and chronic toxicity, *in situ* toxicity, benthic macroinvertebrate structure and Atlantic salmon populations. EEM studies effectively documented Heath Steele's impacts and the progressive and dramatic recovery of aquatic communities in response to reductions in loadings. This paper presents a summary of the EEM results, and illustrates the effective use of EEM in tracking biological responses to environmental controls at metal mines.

Use of Artificial Stream Systems to Assess the Effects of Mining Effluents on Fish. M.G. Dubé[1], D.L. MacLatchy[2], G. Watson[3], J.M. Culp[1], N.E. Glozier[1] and K. Cash[1]. [1]Environment Canada, National Water Research Institute, Saskatoon, SK; [2]Department of Biology, University of New Brunswick, Saint John, NB; [3]INCO Ltd., Safety, Health & Environment, Copper Cliff, ON.

Artificial stream systems have been approved as monitoring alternatives for the Canadian Environmental Effects Monitoring (EEM) Program to assess the effects of industrial effluents (pulp and paper, metal mining) on fish at sites where standard field surveys cannot be conducted. Sites that are confounded by multiple effluent discharges or the presence of historical effects are examples where artificial streams can be used as an alternative approach to field surveys. In addition to assessment of effects, artificial streams can also be used to assess the extent and magnitude of an effect, or for investigation of the causal mechanisms of an effect.

In 2000, we conducted an artificial stream study at a mine site in northern NB to assess the effects of 20% and 80% (v/v) effluent on juvenile Atlantic salmon (*Salmo salar*) and slimy sculpin (*Cottus cognatus*) (northern NB). The 80% effluent concentration represented current discharge levels at the mine site. Exposure to 80% effluent significantly increased liver size and decreased survival, length, and weight of juvenile Atlantic salmon relative to control fish. Exposure to 20% metal mining effluent did not affect salmon survival, length, or weight but did increase liver size. Accumulation of metals (including Zn) in whole fish tissue supported these results showing significantly higher levels in salmon exposed to 80% effluent. Exposure of young-of-the-year sculpin to 80% effluent also resulted in significant decreases in length and weight.

In 2001, a study was conducted at a copper/nickel mine in northern ON to assess the effects of environmentally relevant concentrations of three mine effluents in isolation and in a cumulative effects assessment on juvenile and adult creek chub (*Semotilus atromaculatus*). Essentially three EEM studies were conducted in a single artificial stream study to assess the effects of each effluent on an environmentally relevant fish species independently and in combination. Endpoints measured included growth, condition factor, liver size, gonad size, egg size, reproductive hormone levels, and tissue body burdens for metals. These studies demonstrated that mesocosms are a scientifically sound alternate methodology to field studies and have provided data on the effects of mining effluents on fish that would not otherwise be obtainable in confounded environments.

Investigating Reference Conditions for Benthic Invertebrate Communities in the Sudbury Mining Area. J. Davidson[1], W. Keller[2], G. Watson[3] and K. Somers[4]. [1]Cooperative Freshwater Ecology Unit, Laurentian University, Sudbury, ON; [2]Ontario Ministry of the Environment (CFEU), Laurentian University, Sudbury, ON; [3]INCO Ltd., Safety, Health & Environment, Copper Cliff, ON; [4]Ontario Ministry of the Environment, Dorset Environmental Science Center, Dorset, ON.

Evaluation of the effects of mining on benthic invertebrates can be greatly complicated by the nature of mining activities. Since many mine sites are located at or near the headwaters of rivers or streams it is often difficult or impossible to find suitable upstream reference sites, against which any downstream effects can be assessed. As well, in areas where extensive urban development has followed the development of the mining industry, such as around Sudbury, ON, watercourses receiving mining effluents can also be subjected to various other stresses such as urban runoff and sewage effluent discharges. In such situations the effects of mining need to be assessed against this background of other multiple stresses. Where conventional upstream/downstream comparative approaches are not applicable, an alternate assessment strategy - termed the reference condition approach (RCA) can be used. RCA relies on comparing test sites to a number of reference sites established in a region. In urban areas, some of these reference sites need to reflect the effects of other environmental stresses to permit the isolation of mining effects. In this paper, we characterize the nature of benthic invertebrate communities, and examine the factors affecting community variability, at a large number of reference sites in the Sudbury, ON, mining area. The sites vary widely in the degree to which they are exposed to other anthropogenic influences that can complicate the assessment of mining effects.

The Significance of Dissolved Organic Carbon in Modifying Nickel Speciation and Bioavailability to the Amphipod *Hyalella azteca*. L.E. Doig and K. Liber. Toxicology Centre, University of Saskatchewan, Saskatoon, SK.

While dissolved organic matter (DOM) is known to modify metal bioavailability and toxicity to aquatic organisms, its affect on Ni bioavailability in sediment is largely unstudied. A series of Ni toxicity experiments were conducted as short-term (48 h) water-only tests using the addition of DOM (of various sources and fractions) to evaluate the effects of DOM on Ni bioavailability and toxicity to *Hyalella azteca* under acute exposure conditions. It was found that with the addition of Suwannee River fulvic and humic DOM, whole peat and peat fulvic acid (0 to 30 mg/L), there were no discernable trends in the 48 h LC_{50} data. While it is known that Ni will complex with organic matter, the Ni concentrations required for acute toxicity were likely greater than what could be significantly complexed by the DOM. Work using a miniaturized ion exchange technique demonstrated that, while Ni^{2+} concentrations were unaltered by DOM at test concentrations, a significant proportion of the free Ni was complexed by DOM at lower molar Ni concentrations. Modeling (using MINTEQA2) of the Ni test solutions using EDTA and citrate as ligands (30 mg/L DOM in reconstituted water) generally support these findings. Overall, while Ni speciation can be significantly affected by organic ligands at low total Ni concentrations (i.e., chronic exposure concentrations), at higher concentrations Ni remains largely in the free ion form and hence bioavailable.

Modelling Lead Interactions at the Gills of Rainbow Trout. L. Silk, A. Macdonald, and R.C. Playle. Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Rainbow trout (*Oncorhynchus mykiss*, ~1 g) were exposed to 0.6 μM Pb (125 μg/L Pb) for 3 h in ion-poor water (Ca, Na ~50 μM; pH ~6.5). Complexing ligands (ethylenediamine, citrate, organic matter) or competing cations (Ca^{2+}, Mg^{2+}, Na^{+}) were added to the water. After exposure, gills were removed and analyzed for Pb using a graphite furnace atomic absorption spectrophotometer. From the complexation and competition data, a conditional equilibrium binding constant (*K*) for Pb-gill interactions was calculated (log *K* ~6.0). Conditional equilibrium constants for Ca^{2+}, Mg^{2+}, and Na^{+} binding at the Pb-gill binding sites were also determined (log *K* ~3.4 to 4.0). These log *K* values were entered into the $MINEQL^{+}$ aquatic chemistry equilibrium program, to predict binding of Pb by trout gills - and therefore Pb toxicity to fish - for water of varying ion content and organic matter concentration. Testing of this lead-gill model will be done over the summer of 2001, using water samples collected from across southern Ontario, including Lake Ontario, Lake Huron, and Lake Erie, plus pond and river water.

Importance of Dissolved Organic Carbon and Intestinal Carbonate Minerals in Seawater Acclimated Rainbow Trout (*Oncorhynchus mykiss*) Exposed to Silver and Copper. J.W. Nichols[1], S. Kask[2], M. Ling[2] and R.C. Playle[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Department of Biology, Wilfrid Laurier University, Waterloo, ON.

To replace water lost by osmosis, marine and euryhaline fish drink seawater, making the gut a target for metal interactions. Preliminary experiments generated data on Ag binding to gills and gut of rainbow trout acclimated to 50% strength seawater (17 ppt, 270 mM Cl) and 75% strength seawater (25 ppt, 405 mM Cl) in the laboratory and then exposed to 0.1 µM $AgNO_3$, with or without 10 mg C/L dissolved organic carbon (DOC). The protective effects of DOC, normally important in freshwater, were eliminated in brackish water conditions, and Ag accumulated primarily in the posterior intestine. Also, carbonate minerals (mixed $CaCO_3$ and $MgCO_3$), thought to have an osmoregulatory function, were found in intestines of the seawater acclimated trout. To further elucidate Ag toxicity in brackish water, rainbow trout are being exposed to 0.1 µM Ag simultaneously at various salinities, and to both Ag and Cu (0.5 µM $CuSO_4$) in full strength seawater (33 ppt, 530 mM Cl). During these experiments gill and intestinal systems, along with carbonate minerals, will be analyzed for metal and ion content, to determine the influence of these carbonate minerals on metal interactions in seawater.

Importance of Organic Matter Quality to Metal Toxicity and Accumulation on the Gills of Juvenile Rainbow Trout. M.L. Schwartz[1], P.J. Curtis[2] and R.C. Playle[3]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Okanagan University College, Kelowna, BC; [3]Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Organic matter (OM) in natural waters reduces acute metal toxicity by complexing metal cations, thereby reducing the amount of metal interacting at the gill. To test the effect of OM quality on the toxic effects of metals we concentrated OM from the Experimental Lakes Area near Kenora, Ontario using a portable reverse osmosis unit. We sampled lakes with a range of size, depth and drainage area to get OM of varying qualities; we also tested Suwannee River OM and Aldrich humic acid. We exposed rainbow trout (*Oncorhynchus mykiss*, ~1 g) to 0.5 µM Cu, Cd, and Pb for 3 and 96 h in the presence of different OMs (~2.5 and 5.0 mg C/L).

Cu accumulation after 3 h was most sensitive to OM quality, where the more allochthonous sources reduced gill accumulation to a greater extent than more autochthonous-like OM. After 96 h less Cu toxicity was seen with allochthonous OM compared to autochthonous OM. Pb also appears to be sensitive to OM quality in the same manner as Cu, but Cd is not. The specific absorption coefficient of OM (absorbance at 340 nm per mg C, as an index of aromaticity) correlates well with gill Cu accumulation as well as with Cu toxicity, and therefore provides a simple method of determining OM quality.

Weight-of-evidence Assessments of a Highly Regulated Water System Post Smelter Mine Improvements. G.P. Thomas[1], W. Duncan[2], K. Munro[1] and N. Munteanu[1]. [1]G3 Consulting Ltd., Burnaby, BC; [2]Cominco, Trail Operations, Trail, BC.

Challenges in demonstrating changes in magnitude and extent of effects associated with discharge from a mine smelter in a large flow-regulated river were met by combining assessment techniques and applying weight-of-evidence (WOE) analysis. A combination of biological components with physico-chemical assessments and use of different techniques to assess the same component permitted the elucidation of a range of environmental factors requiring consideration. Biological assessments can detect stresses such as flow alterations, habitat destruction, and over-harvesting of biological resources, in addition to ones chemically induced. The utility of their responses, if not properly understood by the researcher, may therefore be hampered by the very action of confounding influences imposed by the system, particularly those affected by factors in the temporal domain. The mobility and drift of biota further exacerbate the challenge of monitoring change. This paper discusses how weight-of-evidence (WOE) analysis was used to define changes in the magnitude and extent of smelter-related impacts in a complex environment over time. This was accomplished through the integration of several biotic components, with different and known responses to stimuli such as heavy metal effects in addition to natural and other confounding factors in the flow-controlled river. WOE was also used to determine the reliability of the monitoring techniques in addition to the reliability of the components studied. Use of WOE permitted the inclusion of several techniques, including the use of artificial substrates vs. use of natural substrates, to provide a more robust data landscape, provided caveats to each technique (e.g., limitations and biases) were considered.

Problem Formulation for a Large, Complex Ecological Risk Assessment. G. Brown[1], G. O'Brien[2], R. Hull[1], S.M. Swanson[2] and W. Duncan[3]. [1]Cantox Environmental Inc., Calgary, AB; [2]Golder Associates, Calgary, AB; [3]Cominco Ltd., Trail, BC.

Cominco Ltd. is conducting an ecological risk assessment for the off-site area around their Trail, BC lead/zinc smelter. This off-site area may include approximately 80,000 ha in the West Kootenay Region. The three main issues that are being addressed by the Trail ERA are: [1] any chemical or physical constraints on continued environmental recovery that can be attributed to emissions from the Trail smelter, [2] are existing levels of chemicals, which are a result of smelter emissions, high enough to cause direct toxicity or indirect effects to plants, soil invertebrates, wildlife, and aquatic organisms, and [3] will future levels of chemicals in soil, groundwater or surface water, which are a result of smelter emissions, cause direct toxicity or indirect effects to plants, soil invertebrates, wildlife, and aquatic organisms. The first step of the ERA process is the "problem formulation", which identifies chemicals of concern, ecological receptors and exposure pathways for detailed risk assessment. In this ERA, the problem formulation was very comprehensive and involved a "screening level" assessment of effects on terrestrial and aquatic receptors. Potential ecological risks were identified in both the terrestrial and aquatic ecosystems around Trail. However, there were numerous data gaps identified that must be filled before a comprehensive assessment of risks can be conducted.

The Challenges and Intricacies Associated with the Interpretation of Toxicity Tests Data as Part of EEM Programs. S.A. Whiteway. Jacques Whitford Environment Ltd., St. John's, NF.

The past 10 years has seen the advancement and use of marine sediment toxicity tests as part of EEM programs in eastern Canada. Toxicity tests are one component of the sediment quality triad concept that provides a more holistic approach to EEM programs. There are several examples sediment triad concept being applied to baseline data collected in support of future EEM programs. The baseline data collected for some areas in Eastern Canada represents as near pristine conditions as may exist in today's environment. The baseline data in support of the proposed Voisey's Bay mine/mill environmental impact statement was collected from a remote location approximately 350 km north of Goose Bay, Labrador. This area has been exposed to limited human activities and may be as near to a green environment as one may be expected to find today. Amphipod survival, Echinoid fertilization and Microtox® bioassays were conducted on sediments collected from five bays over a two-year period. The need for ancillary data to help in the interpretation of these toxicity tests was clearly demonstrated, as was the limitations of the tests. Some of the toxicity tests produced what could be regarded as "false positive" results, all which could be explained by the presence of natural ammonia and sulfide levels.

Assessment of Lipid Peroxidation and DNA Damage in Soft-shell Clams: *Mya arenaria* at a Copper Mine Tailings Site in Coastal Newfoundland. D. Hamoutene[1], J.F. Payne[1], A.D. Rahimtula[2], B. French[3], G. Veinott[1] and C. Andrews[1]. [1]Department of Fisheries and Oceans, Science Branch, St. John's, NF; [2]Biochemistry Department, Memorial University of Newfoundland, St John's, NL; [3]Oceans Ltd., St. John's, NF.

This study is part of a project examining long-term environmental and ecotoxicological consequences of sub-marine metal mine tailings disposal. The extent of lipid peroxidation (TBARS), DNA damage as well as catalase activity were assessed in *Mya arenaria* collected over two consecutive years from an abandoned copper ore mine and two reference sites. Copper as well as other metal leachates has the potential to produce biological damage through oxidative processes. The Comet assay was used to assess DNA damage and showed no differences between clams from reference and mining sites.

TBARS levels in clams from the mining site were higher the first sampling year but not the second. Catalase activity was higher the second year suggesting that it may have influenced TBARS levels. These results indicate that tailings have the potential to trigger an increase in lipid peroxidation in animals living in mine tailings but this increase may be episodic. Altogether, it would appear that the metal leachate has limited potential to damage lipid rich cell membranes and also poses little if any genetic toxicity risk to soft-shell clams (supported by TSRI, Health Canada).

Alternative Approaches to Deriving Provincial Water Quality Objectives in Ontario. B.W. Kilgour[1], D.G. Dixon[2], M.D. Paine[3], D.J. Spry[4], G. Bowen[5], R. Hall[6] and G. Crawford[6]. [1]Jacques Whitford Environment Ltd., Ottawa, ON; [2]Department of Biology, University of Waterloo, Waterloo, ON; [3]Paine, Ledge & Associates, Vancouver, BC; [4]Environment Canada, Hull, QC; [5]Toronto Region Conservation Authority, Toronto, ON; [6]Ontario Ministry of the Environment, Rexdale, ON.

The standards development branch of the Ontario Ministry of the Environment develops provincial water quality objectives for surface water for the protection of aquatic life and recreation. It has been suggested by industry and the environmental community at large, that should absence of harm in areas where the PWQOs are exceeded be demonstrated, this should then be taken as evidence that objectives based upon only laboratory-based studies, may be overly protective. In our review, we examined several alternatives to enhance the derivation of PWQOs including: screening level approaches, expression of concentrations as bioavailable fractions, development of site-specific objectives and regional-reference approaches based on field data from uncontaminated locations (i.e. absence of harm in an ecosystem community). None of the alternative approaches was ideal, but the use of regional-reference data was further explored with case studies of As and Cu.

Data from 1800+ river sites were taken from the Provincial Water Quality Monitoring Network (PWQMN) database for the years between 1990 and 1995. In total, 354 sites were classified as reference sites on the basis of deviation from an empirical relationship between alkalinity and conductivity. Only a few sites were situated in the southwest of the province, while reference sites were well distributed across the remainder of the province. Site medians for As exceeded the current Interim PWQO of 0.005 mg/L at only once location. Province wide, the 97.5th percentile for background As concentrations was 0.001 mg/L (n=180). Site medians for copper exceeded the PWQO at only 11 soft-water (i.e., <20 mg $CaCO_3$/L) sites. In soft waters, the 97.5th percentile for background Cu was 0.0026 mg/L, which is above the soft-water Interim PWQO of 0.001 mg/L. In hard waters, the 97.5th percentile for background Cu was 0.0028 mg/L, which was below the hard-water Interim PWQO of 0.005 mg/L.

Implementation of the Metal Mining Environmental Effect Monitoring Program. L. Trudel, K. Hedley and S. Ribey. Environment Canada, National EEM Office, Hull, QC.

The amended *Metal Mining Effluent Regulations* (MMER) under the *Fisheries Act* were recently published in the Canada Gazette 1. It will include a requirement for mines to conduct EEM under the authority of the *Fisheries Act*. The proposed EEM program will require mines to monitor fish, benthic invertebrates, water, sediment, effluent and sublethal toxicity to determine if the mining effluent is having an impact on the aquatic environment. The main elements of the EEM program are a fish population survey, a fish tissue analysis and a benthic invertebrate community survey, to evaluate fish, fish habitat and the usability of fisheries resources. This talk will focus on how the EEM program will be initiated, the requirements of the EEM program, associated guidance for the EEM program and will include a timetable for submission of study designs and interpretative reports.

Aquatic Environment Assessment in Support of Remedial Action Planning – Bluebell Mine, Kootenay Lake BC. R. Baker[1], G. Mann[1], N. Sandstrom[2], C. Wilson[2], W. Kuit[3] and B. Donald[3]. [1]EVS Environment Consultants, North Vancouver, BC; [2]Morrow Environmental Consultants Inc.; [3]Cominco Ltd.

The historic Bluebell Mine is situated near Riondel BC on Galena Bay, Kootenay Lake. In 1997 Cominco Ltd. initiated phased terrestrial and aquatic investigations to identify potential environmental and public safety issues, with the view to determining possible site management options for the site. A Sediment Quality Triad study (i.e., synoptic sampling of sediment chemistry, toxicity and benthic community) was undertaken to determine the spatial extent and magnitude of metals contamination in water and sediments in Galena and Bluebell bays, relative to a nearby reference bay.

Sediment concentrations of As, Cd, Cu, Pb and Zn were high in Galena and Bluebell bays, exceeding AELs by up to two orders of magnitude. Despite high bulk sediment chemistry values, water sampling at the sediment-water interface revealed only minor elevations in dissolved Zn at two stations. Similarly, low toxicity (growth and survival of *Chironomus tentans* and *Hyalella azteca*) and minor effects on the benthic community were observed in Galena Bay, suggesting low bioavailability of metals. Coring in shallow areas of Galena Bay demonstrate tailings thickness ranges up to 2.2 m in depth. The results have been used to guide a phased management and remediation program for the site including removal of mine wastes from upslope and foreshore areas. Future work will focus on metals bioavailability and implications for management options of deeper sediments.

Toxicity and the Scientific Basis for Regulation

Session Co-chairs: M.D. Paine and T. Kierstead

What Scientific Basis of Regulations? K. Hedley[1] and K.R. Munkittrick[2]. [1]Environment Canada, National EEM Office, Ottawa, ON; [2]Department of Biology, University of New Brunswick, Saint John, NB.

The goal of environmental regulations is to enhance the protection of the environment. Science-based environmental research and environmental effects monitoring play important roles in verifying that protection measures lead to environmental improvement, and in identifying new issues that require environmental protection measures be taken. Environmental sustainability, with all the baggage that is carried with the term, is the ultimate goal, not protection from all environmental effects. Once an issue is identified that requires enhanced environmental protection, and a decision is made to "manage" the risk through regulatory action, the management of that risk is technology-based. The assumption of technology-based regulations is that less pollution will help to achieve environmental protection goals. Regulations are just one step in the environmental management cycle, and science can play a key role in many of the steps. Science is making its way into federal regulations, and the role of aquatic toxicology within the regulations has evolved substantially over 30 years. In the 1970s, effluents were acutely lethal and receiving environments were markedly degraded. Acute toxicity testing was included in regulations as a target for achieving improvements in the environment, with the assumption that a 10-fold dilution of non-toxic effluent would also protect the receiving environment from chronic effects. Environmental effects monitoring is now included in regulations to help provide information on effects in receiving environments. To have a larger role in regulations we need science to define adverse effects and the relevance of sub-lethal impacts that are seen.

Evaluating the Persistence of Metal-containing Inorganic Substances. P. Doyle[1], D. Mackay[2], D.W. Gutzman[1], Y. Couillard[1], C. Gagnon[3], M. Berci[1] and S. Babonnaud[1]. [1]Environment Canada, Existing Substances Branch, Hull, QC, [2]Canadian Environmental Modelling Centre, Trent University, Peterborough, ON; [3]Environment Canada, Centre Saint-Laurent, Montreal, QC.

Several international initiatives are underway to assess and rank chemical substances for their persistence, bioaccumulation and toxicity, namely the PBT properties. Since most available toxicity data are for acute effects, toxicity is typically evaluated based on acute data and potential to cause chronic harm is assesses indirectly using persistence. Well developed methods are available to evaluate the persistence of organic substances based principally on half-lives for irreversible degradation processes. Inorganic metal-containing substances are problematic because they generally do not produce toxic effects without first undergoing transformation/dissolution reactions, resulting in release of dissolved forms of metal. Even sparingly soluble metal-containing substances release small amounts of such metal in contact with water. It is the persistence of this dissolved metal that is of relevance in hazard evaluation. Since this metal does not degrade it should be considered infinitely persistent.

An alternative approach to evaluating the persistence of metals in the aquatic compartment has been proposed to regulators, which is based on the half-life for removal of metals from the water column by adsorption to settling particulate matter. However, this sedimentation-based approach is not an appropriate measure of persistence since it involves intermedia transfer (water to sediment) only, it is reversible (metal in sediment can be re-mobilized if conditions in overlying waters change), and results are very dependent on water column conditions (especially depth and sedimentation rates). In addition, if this approach is applied for metals it should in principle also be applied to organic substances, since they too may be removed from water by sedimentation. However modeling results indicate that outcomes are unsatisfactory for organic substances, since hydrophobic organic substances with long degradation half-lives (such as PCBs) have relatively short water column half-lives, and so would be unlikely to be found to be persistent using this approach.

Although persistence based on degradation does not discriminate among metal-containing substances, discrimination is achieved when persistence is applied in combination with other hazard criteria. For example, inorganic metal-containing substances with a high acute hazard potential (e.g., soluble compounds of certain heavy metals) can be separated from those that have low potentials (e.g., insoluble alloys, soluble compounds of alkaline earth metals) by applying appropriate acute toxicity criteria. In hazard evaluation systems that do not use chronic effects data, all inorganic metal-containing substances that meet these acute toxicity criteria are considered to have the potential to cause chronic harm. This outcome is appropriate since empirical data indicate that metals that are acutely toxic at low concentrations are usually also chronically toxic at relatively low concentrations.

Revised Protocol for the Development of Canadian *Water Quality Guidelines* for the Protection of Aquatic for Metals - Amendment to the 1991 Protocol. U. Schneider. Environment Canada, National Guidelines and Standards Office, Hull, QC.

The development of national *Water Quality Guidelines* (WQG) in Canada is done by Environment Canada with the help of the provinces/territories under the auspices of the Canadian Council of Ministers of the Environment (CCME). The process is governed by a nationally (i.e., by federal, provincial, and territorial ministries of the environment) agreed-upon protocol, which was first published in 1991. Recently, the CCME's WQG Taskgroup has suspended development and approval of WQGs for metals until the development protocol has been amended to include scientific advancements made over the last ten years. Although we are currently (May 2001) still in the exploratory stage, we anticipate the protocol amendments to include: natural background concentrations, nutrient essentiality; metal speciation and bioavailability, acceptable versus unacceptable toxicity and environmental concentration data, etc. An overview of the status of proposed amendments and changes will be presented, hopefully followed by a discussion with the audience.

Using Tissue Residue-Based Criteria for Water and Sediment. M.H. Salazar and S.M. Salazar. Applied Biomonitoring, Kirkland, WA.

There is a growing body of evidence to suggest that the use of tissue residues as the basis for environmental regulation is a potentially powerful tool. The scientific basis for this argument is that effects only occur after chemical exposure has occurred at sites of toxic action. While there has been considerable progress in identifying these sites for acute toxicity in fish through the biotic ligand model, these sites have not been identified for other species, and no endpoints other than acute toxicity have been adequately characterized. It has been suggested that until these sites and associated effects are better understood, the total body residue can serve as a reasonable surrogate for chemical exposure at sites of action. The rationale for this argument is that the chemical concentrations in the soft body tissues are proportional to the concentration at sites of action. Furthermore, there is less uncertainty in extrapolating tissue residues in the laboratory to tissue residues in the field when using comparable effects endpoints. Differences in bioavailability and matrix interferences associated with measuring chemical concentrations in water and sediment make these comparisons problematic. Weight-of-evidence approaches have attempted to avoid this problem by combining results from different types of chemical analyses in water and sediment with laboratory toxicity tests and assessments of community structure. The missing link in these approaches, however, is the use of tissue chemistry to confirm that exposure has occurred. We developed an exposure-dose-response (EDR) triad, which emphasizes the need to include tissue chemistry results. This EDR approach is consistent with ecological risk assessment in terms of characterizing exposure and characterizing effects. Examples will be given to demonstrate how critical body residues can be used to establish criteria for environmental regulation.

A Standardized Protocol for Transformation and Dissolution of Metals. J.C. Nadeau, J.M. Skeaff, J.C. McGeer, P. King and M. King. Natural Resources Canada, Ottawa, ON.

The new OECD (Organization for Economic Cooperation and Development) test method for measuring the transformation and dissolution (T/D) of metals has been developed for assessing the potential for these substances to produce bioavailable ions in solution and harm to the environment. The T/D protocol will be used to produce data for hazard classification with respect to the aquatic environment within a Global Harmonized System for application to all chemical substances on the market. Hazard classification has significant implications for environmental protection, labelling, transport and market access. This new protocol recognizes that metals and inorganic metal compounds are unique and distinct from synthetic organic substances. The standard protocol is a 7 d test with loadings of 1, 10 and 100 mg of test substance per litre with measurement of total dissolved metal. The concentrations of dissolved metal are then compared to acute toxicity test results for classification purposes. The concept of T/D for metals hazard classification is relatively new and experimental data are incomplete. Therefore, the T/D protocol is subject to a validation process organized by the OECD Test Guidelines Program and will involve the participation of multiple laboratories. We will discuss some of the background in the development of the T/D protocol, summarize results of experimental work to date and examine some of the as yet unresolved issues.

Working Towards a Comprehensive Model for Environmental Hazard Classification of Metals and Inorganic Metal Substances. J.C. McGeer, J.M. Skeaff, P. King, M. King and J.C. Nadeau. Natural Resources Canada, Ottawa, ON.

Within the framework of the OECD, a protocol for measuring the rate and extent of transformation and dissolution (T/D) of solid metals and metal compounds to the bioavailable form in aquatic media has recently been agreed to as a standardized test. The data from this protocol are to be applied in the classification of these substances for their potential hazard to the aquatic environment. Our recent work with metals and alloys has shown that T/D behaviour can be modelled successfully and is dependent on the physical and metallurgical properties of the substances as well as the test media composition. We have shown that a prediction model based on specific surface area can be used to reliably forecast the T/D behaviour of different forms of a substance. Our current work is focused on understanding the bioavailability of dissolved metal mixtures produced in T/D tests with alloys and linking this to their metallurgical properties. As well, studies are examining the effect of different artificial media conditions on both the T/D and the interaction of water chemistry, dissolved metals and bioavailability. The goal is to develop a comprehensive model for metals and inorganic metal substances that will predict transformation, dissolution and toxicity under a variety of different physical and aquatic chemistry conditions for use in hazard identification.

Significance of As Speciation and Dissolved Oxygen Condition on Arsenic Toxicity to Benthic Invertebrates. E.C. Irving[1], K. Liber[1], J.M. Culp[2], R.B. Lowell[2,4], C. Casey[2], Q. Xie[3] and R. Kerrich[3]. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; [2]Environment Canada, National Water Research Institute, Saskatoon, SK; [3]Department of Geological Sciences, University of Saskatchewan, Saskatoon, SK; [4]Environment Canada, EEM National Office, Hull, QC.

Trace element toxicity has been found to vary with speciation, and increase under low dissolved oxygen (DO) conditions. Nevertheless, current *Canadian Water Quality Guidelines* for trace metals such as arsenic do not consider these factors. This study aimed to determine the combined effect of arsenic (As(III) and As(V)) and DO stress on *Hyalella azteca*, *Chironomus tentans* and *Baetis tricaudatus*, and to determine As speciation at high and low DO levels during 10 to 12 d toxicity tests. DO levels were selected that represented adequate and low conditions to *B. tricaudatus* (8 and 6.5 mg/L), *H. azteca* (6.5 and 4 mg/L) and *C. tentans* (6.5 and 1.5 mg/L).

The toxicity of both arsenic species differed between test species: *B. tricaudatus* was the most sensitive; *C. tentans* was the most tolerant; *H. azteca* had intermediatory sensitivity. As(III) was approximately 10 times more toxic to *C. tentans* than As(V), despite a pulsed exposure of As(III) due microbial oxidation to As(V). Under low DO conditions, the lethal and sublethal effects of As(III) and hypoxia on *C. tentans* were additive. This study indicated that both dissolved oxygen condition and arsenic speciation should be considered when setting As water quality guidelines.

Hormesis and Ecological Risk Assessment (ERA) and Risk Management. P.M. Chapman. EVS Environment Consultants, North Vancouver, BC.

Hormesis is generally defined as a stimulatory effect that occurs when a substance, which at high doses results in negative effects (e.g., growth or fecundity inhibition), produces positive effects at low doses (e.g., growth or fecundity stimulation). It differs from arousal because of its high initial baseline. Concentration/dose responses that appear to be hormetic occur not only in relation to chemical stress but also in relation to ecological changes (e.g., the intermediate disturbance hypothesis, the subsidy-stress gradient). And the term hormesis has been linked with risk-risk decisions (e.g., the potential dangers of ground level ozone to humans (morbidity) compared to the protection it affords from ultraviolet radiation; human body mass index related to mortality; etc.) Thus hormetic phenomena are clearly important and need to be considered in risk assessment and risk management. Detailed level ecological risk assessments (DLERA) need to consider the reality of hormetic phenomena, specifically positive, non-linear responses of organisms, communities and ecosystems to physical, biotic and chemical stressors. Changes required to conventional ERA include: an increased number and range of exposures covering both upper and lower bounds, no preconceived assumptions or defaults, exposure-response modeling, possibly no safety factors, determination of the net effect of a stressor on health, upper and lower uncertainty bounds, likelihood of adverse and beneficial effects, and characterization of risk as a range.

Availability and Toxicity of a Complex Mixture of Pollutants in Lacustrine Sediments. É. Pelletier, M. Barthe, M. Bouffard, C. Dévigne and D. Brion. Institut des Sciences de la Mer de Rimouski (ISMER), Université du Québec à Rimouski, Rimouski, Qc.

Increasing laboratory and field evidences have shown major differences between the measured sediment PAH, PCB and metal concentrations and the availability of these toxicants to biological organisms. The toxicity of contaminated sediments is clearly site specific and depends upon the physical and chemical properties of the studied sediments. In an attempt to evaluate the contribution of sediment PAH concentrations to the whole sediment toxicity and find a method to discriminate PAH toxicity from the effects of other toxicants, a series of 5 cores (0.5 to 1.5 m long) where collected in a lacustrine environment exposed to contaminated industrial outfalls for decades. Cores were sub-sampled in sections of 1 to 2 cm, sediment dried, and chemical analysis performed for total PCBs, individual PAHs and individual trace metals (Hg, Cd, Cu, Zn, Pb, ...).

As basic sediment properties (% water, CHN and grain size) varied little between samples but toxicant concentrations changed drastically between cores it became possible to compare the sediment toxicity of samples riche in PAHs but poor in trace metals, or poor in PAHs and riche in metals or in PCBs, or both. The toxicity was tested on the whole wet sediment, and on the available toxicants extracted with pure water or surfactant solutions using Tween 700. Microtox® solid and liquid phase tests, *Selenastrum* microalgal biotest, and haemocyte viability and phagocytosis were among the toxicity bioassays. Preliminary results indicate that PAHs seem tightly linked to sediment in all sampled cores and show very low extractibility and bioavailability. A statistical model is being developed to discriminate the contribution of each toxicant family to the selected toxicity tests. These results contribute to the debate on sediment quality criteria and the use of toxicity bioassays for regulatory purposes.

Use of Ammonia Toxicity Tests to Develop Site-Specific Protective Criteria.
J.M. McKernan[1], A. Partridge[1] and G.R. Craig[2]. [1]TetrES Consultants Inc., Winnipeg, MB; [2]G.R. Craig & Associates Inc., Bolton, ON.

A Watershed Classification process was initiated by Manitoba Environment in 1991 to protect beneficial use of the urban reaches of the Red and Assiniboine Rivers in the City of Winnipeg. In 1991 and 1992, the adequacy of the scientific basis for the proposed ammonia water-quality guideline became an issue, prompting the Clean Environment Commission hosting the hearings to recommend development of site-specific toxicity data for local biota under locally appropriate test conditions. The City committed to developing these data.

In the Toxicity Workstream for the City's "Ammonia Criteria Study", 26 ammonia toxicity tests were completed on 11 different species of aquatic life including 7 fish species and 4 invertebrate species. This is a considerable technical accomplishment when compared with 27 tests completed on 13 species which were found to be technically acceptable in an Environment Canada literature review of world-wide ammonia toxicity tests used to evaluate the toxicity of ammonia and in deriving a national protective criterion for Canada; and 28 tests completed on 12 species accepted by the U.S. EPA in its world-wide literature review of the tests applicable for deriving protective ammonia criteria for the U.S.A.

Of the tests completed, in the present study, 7 can be used directly in the derivation of a locally appropriate chronic-exposure criterion for ammonia. These tests were conducted on 5 fish species and 2 invertebrate species. Each test meets the objectives of being an in-laboratory chronic-exposure test, using ammonia-spiked river water (as opposed to effluent spiked river water), on local species found in the Red and Assiniboine rivers. LC_{20} or EC_{20} values for each species were calculated. Ten acute-toxicity tests were done on three fish species using both North-End Water Pollution Control Centre ("NEWPCC") effluent and ammonia treatments. Resulting LC_{20} values are very similar despite the treatment and have created the basis for derivation of credible, scientifically validated protective criteria for the study region.

Development of a Concentration-Exposure Model to Determine Effects of Ammonia.
D. Morgan[1], A. Partridge[1] and G.R. Craig[2]. [1]TetrES Consultants Inc., Winnipeg, MB; [2]G.R. Craig & Associates Inc., Bolton, ON.

The 3 most un-ionized ammonia-sensitive species assessed in the City of Winnipeg Ammonia Toxicity Study were walleye, catfish and pike. Each of these was tested using a flow-through system, with Red River water dilution. Mortality was measured daily for each 13 or 30 d test. The survival rates for each species' test-population held at various un-ionized ammonia concentrations over the duration of each test were plotted over time. For all 3 species, population follows a first-order decay model, varying with time, for each treatment. An example of the model is:

$$\text{Survival} = \text{initial population } e^{-kt},$$

where: k=daily mortality; t=time in days. This model was calibrated using test data to allow for a prediction of daily survival over time with constant or varying ammonia concentrations. Daily mortality rate (K), however, varies with un-ionized ammonia concentration, and with increasing concentration, the daily mortality rate increased. A statistical model predicting the daily mortality for various concentrations of un-ionized ammonia was developed and is presented.

Statistics and Compliance Decision Frameworks for Single Concentration Toxicity Tests. B. Zajdlik[1] and J. Schroeder[2]. [1]Zajdlik & Associates, Rockwood, ON; [2]Ontario Ministry of the Environment, Etobicoke, ON.

Single concentration tests using *Daphnia magna* and/or rainbow trout are used to determine the compliance status of effluents directly discharged to Canadian waters. Compliance with both federal and provincial regulations requires that mortality in a test of undiluted effluent does not exceed 50%. In a study undertaken for the Ontario Ministry of the Environment, statistics associated with the compliance decision framework were reviewed. False positive and false negative rates in single concentration toxicity test results were calculated followed by the number of replicates and/or test organisms per vessel that would reduce the false positive or false negative rates to below 5%. In addition, a statistically-based decision framework was compared to the compliance decision framework for both the rainbow trout and *Daphnia magna* tests. Results of the study will be presented.

Implications of False Positive and False Negative Rates on Interpretation of Toxicity Test Data. J. Schroeder[1] and B. Zajdlik[2]. [1]Ontario Ministry of the Environment, Etobicoke, ON; [2]Zajdlik & Associates, Rockwood, ON.

A study was undertaken for the Ontario Ministry of the Environment (OMOE) to calculate the probability of false positive and false negative results in single concentration toxicity tests based on both compliance-based and statistically-based decision frameworks. An effluent was deemed "toxic" in the compliance decision framework when lethality exceeded 50% in the undiluted sample. Using the statistical decision framework, an effluent was deemed "toxic" when a significant difference could be detected in survival between the exposed and control groups. These analyses were used to evaluate whole effluent toxicity test data generated at the OMOE aquatic toxicity laboratory. Results of *Daphnia magna* and rainbow trout tests will be discussed in terms of both decision frameworks.

Use and Abuse of Statistics in Environmental Monitoring and Regulation. M.D. Paine. Paine Ledge and Associates (PLA), North Vancouver, BC.

Environmental decisions and management are often based on the statistical significance of field or laboratory monitoring results, sometimes with unintended consequences. First, statistical significance is often equated with environmental significance. An effect of negligible environmental significance may be statistically significant, or an environmentally significant effect may not be statistically significant. The magnitude of effects that can be detected statistically is determined by sample size, sample variances, and the Type I error rate (α), test and transformation used. Therefore, investigators implicitly define environmentally significant or detectable effect sizes, and would do better to explicitly define those effect sizes, and design studies powerful enough to detect them. Second, despite the probabilistic nature of statistics, results are often treated as absolute (i.e., effect/no effect), usually based on using $\alpha \leq 0.05$ to define significance. A difference with p=0.055 would not be considered an effect whereas a similar difference with p=0.045 would. Both p will flip-flop around 0.05, depending on the study methods, tests and transformations used. Consequently, p between 0.01 and 0.10 should be considered roughly equal and "suggestive" of effects. Investigators and regulators should not use statistics as an apparently objective tool to avoid making potentially subjective interpretations or decisions. Observed effect sizes and their confidence limits, relative to effect sizes considered environmentally significant, and not statistical significance, should drive decisions and interpretation.

Biomonitoring, Bioaccumulation and Biomarkers

Session Co-chairs: M. H. Salazar and S.M. Salazar

When is a *Daphnia* Test Not a *Daphnia* Test? D.G. Poirier. Ontario Ministry of the Environment, Standards Development Branch, Etobicoke, ON.

In 1990 the Federal Environmental Protection Service (EPS) developed a series of biological Reference Methods (RM), which would be used in federal and provincial pollution control regulations. These procedures have since been quoted in the *Canadian Environmental Protection Act*, and in the *Ontario Environmental Protection Act* (EPA), and others. These test methods have stood up in court challenges and have been effective tools for monitoring and abating pollution problems.

In Ontario, the EPA require compliance with "end of pipe limits" on toxicity of effluents to *Daphnia magna* and rainbow trout, using RM 14 and RM 13. These methods have very strict ("must") testing requirements for such things as lighting, temperature, loading density, and many others. But what happens when you unintentionally deviate from a "must" testing requirement? In court, these deviations are vigorously challenged, and often with great success (withdrawn charges).

This paper examines the impacts of some real life unplanned "deviations" from must testing requirements in "Biological Test Method: Reference Method for Determining the Acute Lethality of Effluents to *D. magna* (EPS RM 14)." We looked at the effects of severe and minor deviations in lighting, temperature, and loading density on the toxicity of some common reference toxicants (Zn and NaCl), a pesticide, and an effluent sample. All this to answer the question "When is a *Daphnia* test not a *Daphnia* test?"

Estimating Bioavailability Using Thin Films. J.B. Wilcockson[1] and F.A.P.C. Gobas[2]. [1]EVS Environment Consultants, North Vancouver, BC; [2]Simon Fraser University, Burnaby, BC.

The bioaccumulation potential of many lipophilic organic contaminants is highly dependant on the bioavailability of chemical in a given environmental matrix. This relationship has important ecological management implications as the toxic response of an exposed organism is reflected in contaminant body burden. A new solid phase extraction technique, applicable to various environmental media is presented. The technique has the potential to provide an estimate of contaminant bioavailability in water, sediment and soil, thereby providing more useful information than chemical concentrations alone. The method involves application of thin films of ethylene vinyl acetate on glass surfaces. The coated glass surfaces can then be used as solid-phase samplers of contaminated matrices.

Thin films have been applied to various environmental media to determine firstly how feasible the method is and then to measure equilibrium times, reproducibility and absorbing capacity.

Findings to date indicate that the thin films provide an attractive technique to estimate chemical bioavailability without requiring extensive extractions and cleanup. The results suggest that the method could become an effective tool in the ecological assessment and management of contaminated areas.

Effects of Water Hardness and Salinity on the Toxicity of Commercial and Process Water Extracted Naphthenic Acids. A.J. Farwell and D.G. Dixon. Department of Biology, University of Waterloo, Waterloo, ON.

Surface mining and processing of the Athabasca oil sands deposits in northeastern Alberta, Canada results in the production of a significant quantity of process affected water. This study is part of a larger project investigating the effects of Naphthenic acids (NAs) and salinity on a variety of different biota since there is little information available on the effects of NAs and the interactive effects of NAs and salinity. The present study focuses on the interactive effects of salinity (Na_2SO_4 and NaCl) and water hardness on NA toxicity based on 4 or 7 d static renewal tests of larval growth and survival using fathead minnows (*Pimephales promelas*). Larval fathead minnow tests were conducted in reconstituted soft (40 to 48 mg/L), medium (160 to 180 mg/L), and hard (280 to 320 mg/L) water.

Results showed increased or similar toxicity with the addition of Na_2SO_4 and NaCl to commercially available NA (abietic acid), and to the NA fraction extracted from oil sands process affected water (chemical composition unknown). Increased water hardness resulted in a reduction of NA toxicity. High and low molecular weight fractions of organic acids were also tested. The information obtained from this study is critical to the assessment of the interactive effects of NAs and modifying factors such as water hardness and salinity associated with reclamation options such as constructed ponds and lakes.

Determination of Bioaccumulation and Chronic Toxicity (Critical Body Concentrations) of As, Co, Cr and Mn in *Hyalella azteca* Together with a Summary of Seven Other Metals. W.P. Norwood[1], D.G. Dixon[1] and U. Borgmann[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Environment Canada, National Water Research Institute, Burlington, ON.

Total metal concentrations in the environment do not provide accurate estimates of toxicological effects since toxicity is a function of metal speciation and bioavailability. Biological effects are usually a function of bioaccumulation. Whole body concentration should be a better predictor of effects than concentrations in field-collected sediment and water samples. However, in order to evaluate whole-body concentrations, the relationship between bioaccumulation and toxicity must be determined in laboratory-controlled experiments. Bioaccumulation-toxicity relationships of As, Co, Cr and Mn in *Hyalella azteca* were used to determine Critical Body Concentrations (LBC_{50}s and LBC_{25}s) from controlled, chronic (28-d) toxicity tests in the laboratory. Critical Water Concentrations (LC_{50}s and LC_{25}s) were also determined from exposure-toxicity relationships. Mortality Models, which predict mortality based on total body concentration or water concentration, are proposed for each metal. A summary of Critical Body Concentrations and Critical Water Concentrations of 11 metals (As, Co, Cr and Mn plus Cu, Cd, Hg, Ni, Pb, Ti and Zn) provides the means to interpret the potential effect of bioaccumulated metals. Bio-Concentration Factors (BCFs), based on toxicity threshold concentrations, for all 11 metals are also compared.

Contamination by Polychlorinated Biphenyls (PCBs) in the Liver of Belugas (*Delphinapterus leucas*) from the Saint Lawrence Estuary as a Function of their Sex and their Age. C. Dévigne[1], É. Pelletier[1], S. Moore[2], F. Messier[2] and D. Martineau[3]. [1]Institut des Sciences de la MER, Université du Québec à Rimouski, Rimouski, Qc; [2]Centre d'Expertise en Analyse Environnementale du Québec, Laval, Qc; [3]Faculté de Médecine Vétérinaire, Université de Montréal, Sainte-Hyacinthe, Qc.

In this study, concentrations and distributions of Polychlorinated Biphenyls (PCBs) homologues and congeners were determined in the liver of 24 stranded Saint Lawrence belugas. Comparisons were made between females and males as a function of their age. Concentrations of PCB homologues and congeners have shown a very high contamination of the liver of this belugas population. These results are attributed to the continuous circulation of PCBs in their blood due to the presence of PCBs in their food and their blùbber.

A specific transfer was observed for homologues and congeners from mature females (11 to 31 years old) to calves (0 year old) and to juveniles (0 to 1,5 years old) presumably during gestation and/or lactation. Comparisons with males (adults and youngsters) have shown some differences, which might be explained by differences in metabolism and/or in specific bioaccumulation of congeners and homologues within this population. These differences have allowed a partial characterization of the input from the food web and of the PCBs contamination pattern in the Saint Lawrence Estuary.

Can Blood Metallothionein in Fish be a Useful Biomonitoring Tool? M.D. Dutton[1,2] and D.G. Dixon[2]. [1]BEAK International Inc., Brampton, ON; [2]Department of Biology, University of Waterloo, Waterloo, ON.

The great promise of the inducible metal-binding protein metallothionein (MT) remains largely unrealized more than 20 years since it was first proposed as a biomarker for environmental monitoring. Issues of high variability for MT in monitoring studies have begun to be addressed, but the consideration of appropriate organs for study remains poorly understood. In particular, given the central role of the liver in vertebrate metal metabolism, it is not surprising that surveys examining liver MT concentrations are so often equivocal. One organ that has been overlooked for use with MT is blood. This is surprising, given that erythrocyte MT is well studied in mammals. The advantages of erythrocyte MT are multiple, in that blood has great value for non-destructive biomonitoring and that blood can be technically manipulated with great ease. Furthermore, since fish release erythrocytes into circulation as immature cells (reticulocytes) with active protein-synthetic capacity, the potential for MT to be induced in erythrocytes of fish could be greater than is known to be the case for mammals. The research reported here will discuss preliminary developments for the use of fish blood MT as a non-destructive biomarker of metal exposure in fish.

Seasonal Variation in Concentrations of the Metal Binding Protein Metallothionein in Tissues of Wild Female Lake Trout (*Salvelinus namycush*) and White Sucker (*Catostomus commersoni*). J. Werner[1], K. Wautier[2], C.R. Ranson[2], R.E. Evans[2], C.L. Baron[2] and V.P. Palace[2]. [1]Department of Zoology, University of Manitoba, Winnipeg, MB; [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

Metallothioneins (MT) are low molecular weight, metal binding proteins characterized by heat stability, high cysteine content and the absence of aromatic amino acids. Because MTs are induced in the tissues of organisms environmentally exposed to certain heavy metals, they have been proposed for use as early warning indicators. However, recent evaluations of MT have identified several fundamental knowledge gaps that must be addressed before MT can be used as an effective biomarker. One of the areas identified is the lack of information regarding seasonal and reproductive stage influences on metallothionein protein content, particularly in female fish. To address this knowledge gap, liver, kidney, gill and intestine tissues were collected from female lake trout and white suckers in 3 different freshwater lakes at several times throughout a reproductive cycle. Significant seasonal variation in liver and kidney MT content were observed, while gill and intestinal MT content were relatively constant from season to season. Tissues from fish in a lake historically loaded with Cd. An evaluation of the variation in MT from both species in both the reference and Cd treated lakes is given. These data provide additional information required for the use of MT as a biomarker for metal exposures in fish.

Determining the Effects of Methylated Polycyclic Aromatic Hydrocarbons on the Development of Embryo/larval Japanese Medaka (*Oryzias latipes*). S.M. Rhodes, A.J. Farwell and D.G. Dixon. Department of Biology, University of Waterloo, Waterloo, ON.

The Athabasca oil sands (Alberta, Canada) represent one of the world's largest petroleum deposits. Present extraction procedures create large amounts of waste material (fine tailings), which are deposited in maturation ponds. During the maturation process, both naphthenic acids and polycyclic aromatic hydrocarbons (PAHs) become available. Chemical analysis of tailings water shows higher concentrations of methyl/alkyl PAHs compared to unsubstituted varieties. Alkylated PAHs are not listed as USEPA environmental priority pollutants, since little is known about the toxicological effects of the methylated/alkylated PAHs, found in tailings water. This study focuses on determining the toxicological effects of a number of methyl substituted PAHs including; 9,10-dimethylanthracene, 3,6-dimethylphenanthrene, 4,6-dimethyldibenzothiophenene, and 7,12-DMBA, on the development of embryo/larval Japanese medaka (*Oryzias latipes*). In conjunction, the ability of these methylated PAHs to induce blue sac disease in embryo/larval medaka will be examined.

Results demonstrate that methyl PAHs induce a significant toxicological response, which is equivalent or greater than that of the unsubstituted variety. Tests indicate higher instances of blue sac disease in exposed embryo/larva relative to controls. In addition, extracted PAHs from Mildred Lake settling basin, induces a strong toxicological response within embryonic medaka. Responses include decreased hatch length and increased instances of blue sac disease with increasing exposure.

Current Status of Erythrocytes and Reticulocytes for Estimating Growth Rates in Fish. M.D. Dutton[1,2], D.G. Fitzgerald[3] and D.G. Dixon[2]. [1]BEAK International Inc., Brampton, ON; [2]Department of Biology, University of Waterloo, Waterloo, ON; [3]Department of Natural Resources, Cornell University Biological Field Station, Bridgeport, NY.

A need exists for the development of inexpensive, time-efficient, and sensitive indicators to assess growth in natural fish populations. Current methods use nucleic acid synthesis (RNA:DNA ratios) and/or otolith microstructure analysis. Quantification of red blood cell production (erythropoiesis), as a surrogate for protein synthesis, has been proposed as an alternative method worthy of further study. Rapid growth in fishes stimulates erythropoiesis, represented by an increase in the relative amounts of immature erythrocytes (reticulocytes) in proportion to mature erythrocytes, in circulating blood. In our studies, standard methods were used to produce blood smears, individual blood cells differentiated by eye, and counted using a compound microscope.

Experiments with juvenile rainbow trout (*Oncorhyncus mykiss*) and juvenile yellow perch (*Perca flavescens*) revealed strong relationships between reticulocyte production and specific growth estimates. This presentation will also identify further developments for the use of red blood cell indices for growth description. We consider an experiment that delimited the latency of erythropoeisis in juvenile Atlantic salmon (*Salmo salar*) and field evaluations of the relationship between erythopoiesis and growth rate estimates in three species of sunfish (rock bass *Ambloplites rupestris*, bluegill *Lepomis macrochirus*, and pumpkinseed *Lepomis gibbosus*). This technique appears to hold potential for use in estimating growth rate in natural fish populations or in the lab as a non-destructive measure of growth rate. Because erythropoiesis can be quantified with simple, inexpensive techniques and little equipment, it offers great potential for use in developing countries.

Assessing the Chemical Sensitivity of Teleosts Commonly Used in Toxicological Studies. K.L. Teather[1], J.L. Parrott[2], S.C. Courtenay[3] and M. Boudreau[3]. [1]Department of Biology, University of Prince Edward Island, Charlottetown, PE; [2] Environment Canada, National Water Research Institute, Burlington, ON; [3] Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB.

One of the main goals of toxicological studies is to provide information that may be used to assess the potential impact of specific chemicals on living organisms, particularly humans. One problem with using animals as model organisms for bioassays is the difficulty in comparing responses expressed by species that have substantial variation in physiology. In this analysis, we provide information on the sensitivity of twelve teleosts that are commonly used in toxicological research. Specifically, we examined 96 h LC_{50} values for more than 200 different chemicals obtained from published studies since 1980. These chemicals were selected from a larger group and represent those to which both rainbow trout and fathead minnows, the two most commonly used species, were exposed. The other ten species (bluegill sunfish, guppies, mummichogs, western mosquitofish, largemouth bass, coho salmon, Japanese medaka, goldfish, three-spine stickleback, and common carp) were compared to two most commonly used species to determine if there was a consistent pattern of sensitivity among them.

Gastropods as Biomonitors of Habitat Recovery Following Oil-spill and Bioremediation Strategies in Marine and Fresh Water Marshlands. L.E.J. Lee[1], C. Culshaw[1], D. Sotornik[1] and K. Lee[2]. [1]Department of Biology, Wilfrid Laurier University, Waterloo, ON; [2]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS.

Aquatic and pulmonate snails were evaluated for their suitability as biomonitors of habitat recovery following experimental oil-spills in freshwater and marine marshlands. The mystery snail, *Viviparus georgianus* and the mimic pondsnail, *Pseudosuccinea columella*, were used as sediment quality biomonitors for a controlled oil spill experiment at a wetland site along the St. Lawrence River (Ste. Croix, QC) to assess the impacts of crude oil, rates of natural recovery and the efficacy of bioremediation treatments to enhance bacterial degradation of residual oil in the sediments. Similarly, the mud snail, *Ilyanassa obsoleta* and the periwinkles, *Littorina littorea*, were used as biomonitors for a comparable experiment held at a saltmarsh site along the Atlantic coast (Conrod's beach, NS). Sediments from control sites, and oiled sites with or without the application of fertilizers and/or physical aeration treatments, as bioremediation strategies, were evaluated both *in situ* and under controlled laboratory conditions, at various time intervals. Snail survival, growth, and several physiological parameters were evaluated by *in vivo* and *in vitro* means. Depending on the gastropods' feeding habits, snails could be important and ideal "tools" for testing environmental conditions because of their abundance, ease of collection, wide distribution and relatively sedentary nature.

Metals in Municipal Wastewater Dispersion Plume and their Environmental Fate. C. Gagnon and I. Saulnier. Environment Canada, Centre Saint-Laurent, Montreal, Qc.

Environmental impacts of urban wastewater discharge on receiving waters are numerous and inputs of contaminants such as metals can cause toxicity to organisms in receiving waters, as they may be incorporated in the biota. Physico-chemical conditions of the receiving waters influence metal speciation and bioavailability. The objective of this study was to determine the fate of metals in the receiving waters of a major urban effluent discharging to the St. Lawrence river, by determining their chemical forms in the dissolved and particulate phases. Surface water, suspended matter and sediments were sampled at several sites, 0.5 to 40 km downstream of a municipal outfall plume in the river. Total and extractable metal concentrations were determined by ICP-ES/MS and AAS. Physico-chemical parameters such as pH, turbidity, SPM, and dissolved and particulate organic carbon were also measured to characterize the receiving waters and evaluate their influence on the fate and behavior of metals released from the urban effluent. Partitioning of metals between dissolved and particulate phases varies along the effluent dispersion plume and therefore could strongly influence the exposure routes for aquatic organisms that are exposed to various contaminants released from the effluent.

Disruption of Biogenic Amines Levels and their Metabolism in Freshwater Mussels Exposed to a Municipal Effluent. F. Gagné and C. Blaise. Environment Canada, St.-Lawrence Centre, Montréal, QC.

The purpose of this study was to examine the potential effects of municipal wastewaters on the metabolism of biogenic amines. First, surface water extracts were prepared by passing through 100 mL of unfiltered surface water, obtained from upstream/downstream sites along a municipal plume, followed by elution with 1 mL methanol. The effects of these extracts were examined on [1] serotonin uptake by HT_7 human receptors, [2] mono-amine oxidase (MAO) activity, and [3] vitellogenin expression in trout hepatocytes. In another experiments, levels of catecholamines, serotonin with its major metabolite 5-hydroxyindolacetate (5-HIA), and MAO activity were determined in nerve tissues of mussels exposed for 98 d at –1.5 km upstream and 8 km downstream a major urban (about 1.5 million inhabitants) effluent.

The results show that surface water extracts obtained from the municipal plume contained compound(s) that were able to activate human serotonin receptor and were able to induce MAO in hepatocytes. Activation of the serotonin receptor was significantly correlated with the levels of excreted vitellogenin in the extracellular media ($R=0.95$, $p<0.01$) and MAO activity ($R=0.99$, $p<0.01$) in rainbow trout hepatocytes. Moreover, mussels at the downstream site had depressed levels of serotonin and catecholamines and increased levels of MAO activity and 5-HIA in the nerve ganglias. Therefore, municipal effluents release compounds that are capable disrupting the normal metabolism of biogenic amines in freshwater mussels and in the fish. These extracts are also capable of activating human serotonin receptors and MAO in hepatocytes. Because serotonin is implicated in the sexual differentiation process in fish, the spawning process and maturation of oocytes in bivalves, the reduced levels of serotonin in the nerve ganglias and may perturb the reproductive cycle and the sex ratio in oviparous organisms in the St.-Lawrence River.

Identifying Sources of Chemical Exposure and Associated Biological Effects over Fine Temporal and Spatial Scales with Caged Bivalves. M.H. Salazar and S.M. Salazar. Applied Biomonitoring, Kirkland, WA.

In every caged bivalve study we have conducted where cages were separated by a vertical distance of three meters or less, we have shown statistically significant differences in exposure and/or effects. This does not necessarily mean that all of these differences are environmentally significant or that these types of differences should be expected in all locations. The important point to be made with these results is that this approach has the discriminating power to detect such differences, if they occur. While such small spatial differences would not be expected in the horizontal direction, it is reasonable to assume that caged bivalves have the same potential for detecting such differences in the horizontal plane if they were to occur. This type of discrimination is not practical using more traditional methods such as laboratory toxicity tests, adult fish surveys, or mesocosms. Examples will be given from San Diego Bay, California, Port Valdez, Alaska and the Port Alice pulp and paper mill on Vancouver Island, British Columbia. These studies will also be placed in the context of EEM for pulp and paper mills to identify the type of data that can be collected and how the data could be used. The versatility, applicability, and discriminating power of the caged bivalve methodology across a variety of temporal and spatial scales will also be discussed.

POSTER PRESENTATIONS/SÉANCES AFFICHES

Environmental Effects Monitoring - Pulp and Paper and Mining

Metal Contamination Effects on Species Interactions in an Experimental Aquatic Food Web.
D.J. Riddell[1], D.J. Baird[1] and J.M. Culp[2]. [1]University of Stirling, Stirling, United Kingdom; [2]Environment Canada, National Water Research Institute, Saskatoon, SK.

This study seeks to provide an alternative method of effects testing based on the working hypothesis that behavioural endpoints can resolve more subtle responses to environmental contamination than acute and chronic tests on single species. The foraging and predator-avoidance behaviours of mayfly (*Baetis tricaudatus*) nymphs and predator-prey interactions were investigated under differing combinations of toxicant concentration and predation risk. Predators consisted of stonefly (*Kogotus nonus*) nymphs, juvenile brook trout (*Salvelinus fontinalis*) and longnose dace (*Rhinichthys cataractae*), either alone or in combination. Organisms were exposed to 0, 0.5 and 5.0 µg/L Cd for 7 d (invertebrates) or 30 d (vertebrates) prior to observation in artificial stream channels. Behavioural observations (20 min duration) were made at 09:00, 15:00, 21:00 and 03:00. Mayfly feeding rate was assessed by image analysis of grazed diatom mats cultured on unglazed ceramic tiles.

The range of Cd concentrations investigated had no significant effect on the foraging and predator-avoidance behaviours of mayfly and stonefly nymphs. However, the presence of predators had significant effects on invertebrate behaviour. Mayfly drift rate increased in the presence of stoneflies and decreased in the presence of trout. Trout and dace also significantly decreased both the number of mayflies present on tile surfaces and the time spent active by stoneflies. Compared to controls, 5.0 µg/L Cd-exposed trout and dace exhibited significantly reduced capture efficiencies and were more active at this concentration. Thus, capture efficiencies and prey behaviours appear to have the potential to indicate subtle, sublethal effects of Cd contamination.

Do Mine Tailings Entering Wabush Lake Affect the Endocrine Function of the Pituitary of White Sucker (*Catostomus commersoni*)? G.E. Fåhræus-Van Ree[1] and J.F. Payne[2]. [1]Department of Biology, Memorial University of Newfoundland, St. John's, NF; [2]Department of Fisheries and Oceans, St. John's, NF.

Although most studies to date on endocrine disruption in fish have emphasised steroid hormone linked perturbations, other hormones such as peptides, which play a critical role in growth and developmental processes, may be important targets. The histological and immunocytochemical characteristics of the pituitaries of white sucker were examined in fish caged in Lake Wabush, which receives a high volume of iron ore tailings, and in an adjoining reference lake, Lake Shabogamo. Studies carried out earlier by ICP-MS on levels of metals in the lakes demonstrated a strong gradient. Fish approximately 11 to 13 cm in length were placed in 20 L perforated buckets (15 fish per bucket) which were anchored at a depth of 3 meters below the surface of each lake for two weeks.

Immunochemical reactions as measured by amount and intensity of fluorescence were significantly different ($p<0.02$) in the acidophilic somatotrophs of fish from Wabush Lake

(0.009±0.008 24.2±20.6 AU/area, respectively) in comparison with Shabogamo Lake (1.254±0.025, 273.2±72.0 AU/area). No differences were obtained for the basophilic gonadotrophs. The water quality of Wabush Lake may be impaired to a sufficient extent to negatively affect the synthesis of growth hormone in fish and potentially growth and development (Supported by the ESSRF research program, DFO. We also thank Lee Preziosi, Iron Ore Canada for support with our studies).

Effects of Naphthenic Acids and Salinity on Cell Lines Derived from Rainbow Trout (*Oncorhynchus mykiss*). L.E.J. Lee[1], K. Haberstroh[1], D.G. Dixon[2] and N.C. Bols[2]. [1]Department of Biology, Wilfrid Laurier University, Waterloo, ON; [2]Department of Biology, University of Waterloo, Waterloo, ON.

Naphthenic acids (NAs) are complex mixtures of saturated carboxylic acids found at high levels in oil-sands tailings. The exact chemical composition of NAs is not known and their effects on biota have been poorly investigated. Reclamation of oil from the oil-sands in Alberta has led to environmental concerns regarding its extraction procedures as large volumes of tailings are released into the environment. The present study was designed to investigate the cytotoxic effects of crude NAs prepared from a settling basin and from commercially purchased NAs, as well as to develop rapid cost-effective bioassays to quantify NA mixture toxicity. Rainbow trout cell lines derived from gill, liver, spleen and gonads were examined for impairment in several cellular functions using a fluorescence microplate reader. Cell membrane integrity, mitochondrial activity and lysosomal function were monitored at various NA and salinity concentrations and time points. Salinity appeared to enhance the toxicity of NAs. This approach could provide a reference base for rapid assessment of NA toxicity as well as from its fractions and mixtures to biomonitor oil constituents and contribute to environmental risk assessment. (Supported by TSRI)

The Effects of Pulp Mill Effluent on Benthic Invertebrate Communities in the Atlantic Region (Cycle 2 Environmental Effects Monitoring). K.K.J. Kim[1] and W.R. Parker[2]. [1]Environment Canada, Environmental Protection Branch, [1]Dartmouth, NS; [2]Environment Canada, Environmental Protection Branch, Fredricton, NB.

The Pulp and Paper Effluent Regulations (PPER) under the *Fisheries Act* require all mills to conduct an environmental effects monitoring (EEM) program on a regular basis. The objective of EEM is to assess the adequacy of PPER for protecting fish, fish habitat and the use of fisheries resources. Benthic invertebrate community studies were conducted by all mills in the Atlantic region to assess the effect of pulp mill effluent on fish habitat. The data indicated a significant improvement in the benthic communities at most of the mills in the Atlantic region after the installation of secondary treatment. The results also indicated that benthic invertebrate community studies are relatively sensitive and effective in detecting subtle changes in benthic environmental conditions. An overview of the data analysis used and results will be presented.

Toxicity and the Scientific Basis for Regulation

Ecological Risk Assessment: A Promising Approach to Assess Metals Contamination Caused by Mining Discharges in Abitibi, Quebec. L. Parent and L. Vescovi. Téléuniversité,

Université du Québec, Montréal, Qc.

Allowing the integration of the concepts of sustainable development which aims at a harmonious and respectful socioeconomic growth of the environment, ecological risk assessment is presently a scientific discipline rapidly expanding. The recent evolution of ecological risk assessment studies aims towards an application and an integration in environmental management as in the governmental institutions as in the private sector. The procedure of ecological risk assessment developed by the US-EPA appears as being a reference in the domain. It widely inspired the Canadian Council of Ministers of the Environment (CCME) during the elaboration of its framework for ecological risk assessment. In Quebec, it inspired the procedure of ecotoxicological risk assessment for the rehabilitation of the contaminated sites. The potential of this discipline are real, they open to the scientific community completely new innovative fields of investigations. On the basis of a review of the literature, this presentation aims at first to draw up a survey in ecological risk assessment. It confirms the absence of the ecological risk assessment studies applied to the aquatic environment in Quebec. Secondly, it raises the new scientific challenges in this domain in the province. Further to an analysis of the situation and needs, proposed areas of research concern the adaptability of the studies of ecological risk assessment in evaluation of the ecological effects of: [1] multiple stressors on the watershed scale, [2] the mining discharges, and [3] urban discharges.

Lethal and Sublethal Effects of DDAC on Early Life Stages of White Sturgeon (*Acipenser transmontanus*). C. Wong[1], S.J. Teh[2] and V. Furtula[3]. [1]Environment Canada, Aquatic and Atmospheric Sciences Division, Vancouver, BC; [2]School of Veterinary Medicine, University of California, Davis, CA; [3]Environment Canada, Pacific Environmental Science Centre, North Vancouver, BC.

The study objectives were to quantify the concentrations causing mortality and impacts on growth during and after exposure of white sturgeon (*Acipenser transmontanus*) to didecyldimethylammonium chloride (DDAC). Four early life stages (3-, 11-, and 42- and 78-day-old) were exposed for 96 h using the standard Environment Canada protocol (EPS 1/RM/9; 1990) for acute toxicity testing. Surviving fish were transferred to clean well water and raised for 18 to 21 d for growth determination.

The results showed age- and concentration- dependent responses. 96 h LC_{50} DDAC values were 10.0 to 50.0 (3-day-olds), 58.5 (11-day-olds), 99.7 (42-day-olds), and 100.0 to 250.0 (78-day-olds) µg/L. Body lengths and weights of white sturgeon that survived exposure to DDAC were lower than controls of the same age. After 18 to 21 d in clean well water, survival was 2 times lower and growth was 2 to 3 times lower in treatment groups than controls of the same age.

Based on the results, the *Interim Canadian Water Quality Guideline* of ≤1.5 µg/L DDAC is adequate to protect white sturgeon from acute DDAC lethality. While the guideline is more stringent than the lowest DDAC concentration tested, the fact that growth impairment and post-exposure morbidity was observed suggests that chronic exposure at the guideline level could result in effects.

Risk Assessment of 25 Priority Substances in Canada: Approach and Outcomes. P.W. Harris and N. Davidson. Environment Canada, Existing Substances Branch, Hull, QC.

In Canada, the *Canadian Environmental Protection Act* (CEPA) requires the Ministers of the Environment and of Health to establish a Priority Substances List (PSL), which identifies substances to be assessed on a priority basis to determine whether they pose a significant risk to the environment and to the health of Canadians. In December 1995, 25 substances including individual chemicals, mixtures, and effluents were added to the PSL. The purpose of the assessments is to determine whether or not a substance is "toxic" as defined by CEPA. Under CEPA, a substance is considered "toxic" if it enters or may enter the environment in amounts or under conditions that may pose a risk to human health, the environment or its biological diversity, or to the environment on which life depends. Thus "toxic" in the context of CEPA is a function of both the inherent properties of a substance and of the amounts, concentrations, or nature of entry of the substance in the Canadian environment. Environment Canada is responsible for examining the risk these substances may pose to the environment, and therefore have developed an approach for conducting ecological risk assessments. Results of the 25 risk assessments are now available.

Follow-up Report on the First Priority Substances List Environmental Risk Assessment of Short-chained Chlorinated Paraffins under the *Canadian Environmental Protection Act 1999* (*CEPA 1999*). W. Windle[1] and R. Sebastien[2]. [1]Environment Canada, Hull, QC; [2]Pest Management Regulatory Agency, Health Canada, Ottawa, ON.

The term "chlorinated paraffin waxes" is generally restricted to chlorinated paraffins having long carbon chains ($C_{18\text{-}28}$). However, the scope of the assessment was broadened to include the short chain ($C_{10\text{-}13}$) and medium chain ($C_{14\text{-}17}$) chlorinated paraffins which are also of concern because of their potential effects on the environment and human health. Chlorinated paraffins (CPs) are produced in, and imported into, Canada for use as plasticizers and flame retardants as well as extreme-pressure additives in lubricating oils. They are persistent compounds and have the potential to bioaccumulate in aquatic organisms. Short chain chlorinated paraffins cause adverse effects in fish and aquatic invertebrates at concentrations below 1 μg/L in laboratory studies. The conclusion of the 1993 environmental risk assessment of Chlorinated Paraffins was that there were no data identified on the concentrations of these substances in any medium in the Canadian environment on which to assess whether these compounds are "toxic" as defined under the *Canadian Environmental Protection Act (CEPA)* [1988]. Further research was recommended to generate new scientific information on levels of short-chain chlorinated paraffins (SCCPs) in various wastewater treatment effluents from southern Ontario, Canadian lake sediments, Lake Ontario fish and Lake Ontario water. Results of this research and conclusion under the revised CEPA, CEPA 1999, are presented.

Proposed *Canadian Water Quality Guidelines* for Nitrate. J.D. Whall[1], K.L. Potter[2], E.S. Roberts[2], D.J. Spry[2] and P.-Y. Caux[2]. [1]J.D. Whall Environmental, Ottawa, ON; [2]Environment Canada, National Guidelines and Standards Office, Hull, QC.

Increasing nitrate concentrations in Canadian waters have prompted an examination of potential biological impacts of this nutrient. Although naturally occurring, nitrate may also be introduced into the environment in large quantities through fertilizers, wastewater effluents, and sludge disposal. Other nitrogenous substances released to the environment through anthropogenic sources, such as fossil fuel combustion, may also be converted to nitrate through microbial processes. Nitrate salts are often used for the toxicity testing of metals due to the high solubility and low toxicity of the nitrate anion. Nevertheless, toxic effects of the nitrate anion have been observed in fish, amphibians, and invertebrates in fresh and marine waters. *Canadian Water Quality Guidelines* (CWQGs) for the protection of aquatic life are being developed for nitrate based on nationally approved protocols. CWQGs are nationally endorsed, science-based levels that should result in negligible risk to biota and ecosystem health. These guidelines are benchmarks of environmental quality that can be used for the protection, sustainability, and enhancement of the Canadian environment and its beneficial uses. Complex issues were considered in the development of these nitrate guidelines, including: the influence of associated cation used in toxicity testing; the complexity of the nitrogen cycle; plant/algal nutritional requirements; and nitrogen-to-phosphorus ratios and their effects on eutrophication. Proposed nitrate CWQGs are also compared to those from other national/international jurisdictions.

Toxicity Testing Methods - Development and Applications

Trophic Transfer and Immunotoxicity of Tributyltin in Boreal Starfish *Leptasterias polaris* (Echinodermata, Asteroidea). K. Békri and É. Pelletier. Institut des Sciences de la Mer de Rimouski (ISMER), Université du Québec à Rimouski, Rimouski, Qc.

Tributyltin (TBT) is an environmental toxicant which has detrimental effects on immune system of numerous aquatic organisms. This study sought to investigate the potential *in vivo* immunotoxic effects of TBT, after trophic transfer, on amœbocytes phagocytosis of the estuarine starfish *Leptasterias polaris*. To our knowledge, this is the first study assessing immunotoxic effects of TBT on echinoderms. Tested animals were contaminated by trophic transfer via blue mussels exposed during 24 h in water containing TBT (3 mg TBT/kg wet weight tissue). Four biomarkers of immunotoxicological effects were monitored over 45 d at different incubation times (9 h, 24 h, 48 h, 72 h, 11 d, 18 d, 25 d, 32 d and 45 d) : amœbocytes count, and cell membrane injury or viability using trypan blue exclusion test assessed with light microscope, phagocytic activity measured with an epifluorescence microscope using a suspension of dead bacteria labelled with fluorescein isothiocyanate injected directly in the cœlomic fluid of the animals, and lysosomal integrity using neutral red retention measured with a spectrophotometer.

Data showed that TBT and it's metabolites, mono- and di-butyltins, accumulated preferentially in pyloric caeca, and gonads contained only small amounts. Despite the differences in exposure periods to the contaminated diet, in burdens of butyltins ingested between the various contaminated groups, and the depuration period (one week), there is no significant differences in body burdens of butyltins detected in analysed contaminated starfish tissues. Results of exposition biomarkers showed that the boreal starfish possess necessary mechanisms to depurate the ingested butyltins, thus playing role of "recycling organism" of butyltins into the environment. In spite of no detecting butyltins in cœlomic fluid, there effects have been reflected on the starfish phagocytic activity and lysosomal retention, with no effect detected on amœbocytes count and membrane integrity.

Study of the Chemical Sequestration of Polycyclic Aromatic Hydrocarbons (PAH) by Selective Extraction of Lacustrine and Marine Sediments. M. Barthe and É. Pelletier. Institut des Sciences de la Mer de Rimouski (ISMER), Université du Québec à Rimouski, Rimouski, Qc.

The objective of the project was to develop a selective extraction method for Polycyclic Aromatic Hydrocarbons (PAHs) by using surfactants to understand the influence of sequestration on the bioavailability and the toxicity of the PAHs present in the sediments of a lacustrine environment (Saint-Louis River, Qc) and a marine environment (Saguenay Fjord, Qc). To determine the optimal surfactant and the optimal molarity of that surfactant which would be used to calculate the PAHs sequestration, several tests have been settled on a number of commercial surfactants with variable polarity (Brij®, Tween®, AOT). The results of this screening show that the optimal surfactant was the polyoxyethylene(100)stearyl ether (Brij® 700) with an optimal molarity of 5.25×10^{-3}/mol. In parallel, physical and chemical characteristics of tested sediments (granulometry, organic carbon content, C/N, trace metals, PAHs, PCBs) were determined. The exhaustive extraction of PAHs was performed with dichloromethane (DCM) providing a basis to calculate the extraction capability (%) of each surfactant tested and thus estimate the chemical sequestration (%) of each PAH extracted. In order to estimate the bioavailability of PAHs in bulk sediment and in extracted solutions, their toxicity was determined by a series of microbiotests including Microtox® solid and liquid phases, microalgae, and phagocytosis. Combining the results of chemical and biological tests, it will be possible to establish a correlation (positive or negative) between the extractability (%) of PAHs and the global toxic response of extracted compounds. It will be possible to compare the sequestration (%) for the Saint-Louis River and the Saguenay Fjord sediments and to determine if the marine environment has an influence on the sequestration and the potential toxicity of these sediments.

Implementation of Sediment Quality Criteria with Respect to Background Metal Levels in Post Glacial Marine Clays from the St. Lawrence River. I. Saulnier, C. Gagnon, S. Lepage and L.-F. Richard. Environment Canada, Centre Saint-Laurent, Montreal, Qc.

Pre-industrial marine clays, often dredged in the maritime seaway, represent a concern for the environment management as they are considered potentially toxic with regards of the sediment quality criteria. A research project was initiated in 1999, at the St.-Lawrence Center, to better document the background levels of target chemicals in these "uncontaminated" sediments. Over 100 stations were sampled with corers in 1999 and 2000. Three different extractions were performed to evaluate the distribution and potential bioavailability of trace metals: a total extraction, which included HF dissolution, a total recoverable extraction with nitric and HCl concentrated acids, and a weak digestion with 1N HCl.

Preliminary results show that Cr and Ni concentrations in these sediments exceed the level of severe effects to the living organisms when solubilised by strong acids whereas their predicted toxicity decreases with less aggressive digestions, reaching the no-effect level with a 1N HCl extraction. Other trace metals, such as Cu, Pb and Zn also show the same relation, although less contrasting. These results highlight the association of Cr and Ni to the inert silicate fraction of marine clay sediments, whereas Cd, Pb, Zn as well as Cd and Hg display generally an affinity towards more reactive phases of the sediment. Normalization of trace metal concentrations with organic carbon content, grain size distribution and conservative elements such as Al and Li were also investigated for a better application of the criteria to pre-industrial marine clays.

Effects of Water Source on Metal Bioavailability and Toxicity from Field Collected Sediments. M. Nowierski[1,2], D.G. Dixon[1] and U. Borgmann[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Environment Canada, National Water Research Institute, Burlington, ON.

The toxicity of sediments to *Hyalella azteca* is a function of the amount of metal accumulated and not on the concentration of total metals in sediment. Metals responsible for toxicity can be identified by comparing metal bioaccumulation to critical body concentrations. Standard sediment toxicity tests use laboratory water as overlay water. Lake water chemistry (hardness, pH, DOC, etc.), however, is highly variable and is known to affect metal speciation, binding to sediments and toxicity. The release of metals from the sediment, and the toxicity of those metals once in solution, could therefore be affected by overlying water chemistry. The objective of this study is to compare metal bioavailability and toxicity in laboratory toxicity tests with field collected sediments using both standard laboratory water and natural lake waters.

Preliminary 4 week chronic toxicity tests were completed using sediment from three sites in each of ten lakes in Rouyn-Noranda, Quebec with Lake Ontario overlay water in order to identify lakes with high metal bioavailability in their sediments. Reduced survival and growth of *Hyalella azteca* resulted following exposure to sediment from one of the test lakes (Lac Dufault). Bioaccumulation of Cd exceeded the critical body concentration known to cause toxicity (LBC_{25}), therefore these effects were attributed to Cd. Lakes for further study will be selected based on metal bioavailability in the sediment and a wide range of lake-water chemistry. Toxicity tests will be conducted with both laboratory and site water for each sediment.

Solution Replacement–avoid Overstating Toxicity of De-icers to Fish. G.R. Craig[1], D. Lester[2], R. Blessing[2] and M. McGuinnes[2]. [1]G.R.Craig & Associates Inc., Bolton, ON; [2]The Dow Chemical Co.

The aquatic toxicity testing protocol 40 CFR 797.1400, used to assess commercial products, can overstate the toxicity of readily biodegradable products. When products are tested under warm (22°C) fathead minnow test conditions, the four-day exposure period can exceed the bacterial acclimation period and oxygen concentrations can be depressed. Tests conducted on the same product at the cooler rainbow trout (12°C) test temperature are complete before bacteria can acclimate and oxygen remains relatively stable.

Tests of a single de-icer demonstrate that static tests conducted at 22°C produce a more toxic result when oxygen is depressed than when tested under 12°C conditions when oxygen is more stable. Aeration of the test is not permitted and the more expensive continuous flow exposure is the only alternative to static testing presented in the protocol. Daily solution replacement with static test systems maintains de-icer concentrations and oxygen levels, prevents bacterial acclimation and toxicity results are more similar for both temperatures and fish species.

Accumulation of Tributyltin in *Hyalella azteca* as an Indicator of Chronic Toxicity: Survival, Growth, and Reproduction. A.J. Bartlett[1], D.G. Dixon[1], R.J. Maguire[2], S.P. Batchelor[2] and U. Borgmann[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Environment Canada, National Water Research Institute, Burlington, ON.

Body concentrations of contaminants have been shown to be more reliable indicators of toxicity than water or sediment concentrations. There are numerous advantages to this approach: body concentrations are independent of variable test/field conditions (e.g., pH, complexing agents), comparisons of sensitivity can be made between species, and only the amount of bioavailable contaminant is measured. Previous studies have determined the body concentration:toxicity relationship for tributyltin (TBT) in *Hyalella azteca* collected from laboratory bioassays, and used this relationship as a tool to predict TBT toxicity to amphipods exposed to sediments collected from the field. However, these were water-borne exposures, while exposure to TBT in the field occurs via sediment as well as water. Furthermore, the exposures were only 4 weeks in duration, which is too short a time to measure reproduction, a key endpoint in TBT toxicity. In order to address these deficiencies, spiked-sediment experiments were carried out over two generations of *H. azteca*. The validity of each method was evaluated by comparing: [1] 4 week LC_{50}s of water and sediment bioassays, [2] body concentrations at the end of 4 and 10 week exposures, and [3] body concentrations of first generation and second generation animals.

Testing of Water Availability for Polymers Submitted for Assessment Under the *Canadian Environmental Protection Act* (CEPA). D.J. Porter, R. Lawuyi, S. Falicki and A. Atkinson. Environment Canada, Hull, QC.

The *Canadian Environmental Protection Act* (CEPA) requires that all new substances be assessed for "toxicity" as defined by legislation. Notifiers of new substances for the Canadian market are required to provide detailed information and test data prior to the commencement of any manufacture or import. For polymers, one of the key data elements to be submitted is water solubility at different pHs. However, the complexity of polymers with a wide range of molecular weights makes the water solubility determination of some polymers difficult and may not capture the amount "available" to aquatic organisms. Therefore, industry and government have identified deficiencies with certain methods that are used to obtain water solubility data for polymers. The recommendations for appropriate methods of measurement of water availability, sample preparation and standardized test media for polymers submitted to the New Substances Program under CEPA are discussed.

Toxicity of Uranium Over the Life Cycle of the Aquatic Invertebrate *Chironomus tentans*. J. Muscatello, K. Liber and S. Stoughton. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK.

Northern Saskatchewan is home to some of the top-producing uranium (U) mines in the world. The aquatic ecosystems are often the major systems affected by the release of U, especially from process effluents. Here, benthic invertebrates are likely among the most highly exposed organism due to the capacity of sediments for accumulating U. Aqueous U toxicity has been investigated in a number of aquatic invertebrates. However, limited data are available on the effects of U exposure on benthic macroinvertebrates, especially regarding reproduction, which is one of the most relevant endpoint for ecological risk assessment. The objective of this work is to evaluate the effect of U exposure over the entire life cycle of the aquatic invertebrate *Chironomus tentans*. A static-renewal test is being performed using carbon-filtered water spiked with one of four different U concentrations (0.04, 0.2, 1 and 5 mg/L), which will be replaced every 2 d. The test was started with first-instar larvae in 300 ml test beakers containing a thin layer of silica sand.

Food is added everyday. Throughout the study, larval, pupal and adult survival, U tissue concentration, mating success (between exposed and unexposed organisms), hatching success (of the F_1 generation) and maternal U transfer to the offspring will be evaluated. This two month long experiment, initiated in May 2001, will help to better understand U toxicity for the protection of aquatic environments focusing on ecologically relevant endpoints such as development, reproduction and fecundity.

Toxic Actions of Alkylated Naphthalenes to Rainbow Trout Cells in Culture. V.R. Dayeh[1], G. Jeremic[1], K. Schirmer[1], J. Lew[1], S. Lee[1], P.V. Hodson[2] and N.C. Bols[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Department of Biology, Queen's University, Kingston, ON.

A liver cell line, RTL-W1, and a gill cell line, RTgill-W1, from rainbow trout were used to investigate the toxic actions of 12 substituted naphthalenes: 1 methyl, 2 methyl and 10 dimethyls at positions, 1,2; 1,3; 1,4; 1,5; 1,6; 1,7; 1,8; 2,3; 2,6; 2,7. For an aryl hydrocarbon receptor-mediated mechanism, the induction of 7-ethoxyresorufin-o-deethylase (EROD) activity was investigated in RTL-W1. None induced EROD activity. For cytotoxicity, three fluorometric assays of cellular function were investigated with RTgill-W1. These were alamar Blue (AB) for metabolic activity, CFDA-AM for membrane integrity and neutral red (NR) for lysosomal activity. The detection of cytotoxicity depended on how the cell culture wells were dosed. Little or no cytotoxicity was observed when the compounds were first mixed into the medium followed by the addition of the mixed medium to the cells. However, cytotoxicity was observed consistently when the compounds were dissolved in dimethylsulfoxide (DMSO) and the DMSO solution was added directly to the medium within culture wells. The difference is attributed to retention of test compounds on vessel surfaces during mixing and to DMSO enhancing cellular uptake. The three cytotoxicity assays gave similar results. The substitution pattern appeared to influence toxicity, but substituted naphthalenes were not substantially more toxic than the parent compound, naphthalene.

Acute Toxicity and CYP1A Induction Capacity of Pulp Mill Biosolids to Rainbow Trout. S.A. Hawkins[1], R. Wehrell[1], L. McCarthy[2], M.R. van den Heuvel[3] and P.V. Hodson[1]. [1]School of Environmental Studies, Queen's University, Kingston, ON; [2]Ryerson University, Toronto, ON; [3]Forest Research Institute, Rotorua, New Zealand.

Pulp mill biosolids and biosolid extracts were tested to determine if the chemicals causing toxicity of pulp mill effluent, removed during primary and secondary treatment processes, were instead transferred to the particulate and organic matter comprising biosolids. Activated sludge from a thermomechanical pulp mill was tested for lethality and CYP1A induction using a 96 h rainbow trout (*Oncorhynchus mykiss*) bioassay.

Direct biosolid exposure produced an LC_{50} of 0.44 g ww/L, and there was no evidence of increased CYP1A activity in fish surviving sub-lethal concentrations (<1 g ww/L). Testing with methanol and aqueous extracts of dried biosolids was conducted to identify the hydrophobicity or hydrophilicity of the toxic constituents, and thereby provide some information regarding the partitioning mechanism. After normalizing to equivalent weights of biosolids, the aqueous extract was less toxic than the parent material and did not increase CYP1A activity. In contrast, the methanol extract was highly toxic, and the CYP1A activity increased linearly with exposure. These preliminary findings suggest that the toxic constituents removed from pulp mill effluent are not entirely mineralized, but remain present and bioavailable in biosolids, are relatively hydrophobic, and present a toxicity hazard to fish.

Development of a Proteomics Approach for the Evaluation of Cytotoxic Effects using Cells in Culture. L.E.J. Lee, C. Culshaw and S. Willfang. Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Proteomics, or the qualitative and quantitative analysis of total cellular proteins, is an old technology with a new name enhanced with the availability of powerful computers, appropriate software and large databases readily accessible via the Internet. The field of expressional proteomics allows mass screening of proteins from differing sources and quantitative measurements of its changes in response to biological perturbations such as disease, hormonal or drug treatments and response to toxicants. Observation of changes at the protein level, unlike genomics, allows for functional analysis of cells and organisms, and the determination of mode of action at the cellular level. A proteomics approach to evaluate changes in trout cell lines in response to contaminants has been initiated. Protocols for obtaining a protein fingerprint database (proteome) of various rainbow trout cell lines under normal and toxicant exposure using 2D gel electrophoresis and computerized image analysis will be presented. This could provide insights into the mechanism of toxicant action by identifying proteins whose expression is modulated by toxicant exposure in fish. (Supported by NSERC)

The Effects of Oil Sands Compounds on the Early Life Stages of Fathead Minnows. M.V. Colavecchia[1], S.M. Backus[2], P.V. Hodson[1] and J.L. Parrott[2]. [1]Department of Biology, Queen's University, Kingston, ON [2]Environment Canada, National Water Research Institute, Burlington, ON.

This study examines the effects of exposure to naturally derived oil sands compounds on the early life stages (ELS) of fathead minnows (*Pimephales promelas*). Sediments were collected from sites along the Athabasca River, Alberta, which are within and outside natural oil sand deposits. ELS toxicity tests (static daily renewal) were conducted using river bitumen deposits, reference and downstream river sediments, controls and refinery pond sediments. Fifteen fertilized eggs were placed in glass containers with a range of sediments, 1 L of dechlorinated water and moderate aeration. Eggs and larvae were checked daily for mortality, hatching and deformities.

Results show significant dose-dependent increases in egg and larval mortality with embryos exposed to natural oil sands bitumen. Larval deformities were significantly higher in embryos and larvae exposed to bitumen as compared to controls and reference sediments. Deformities included edemas, hemorrhages, pigment alterations and spinal malformations. Refinery pond sediments were extremely toxic to larvae, causing significant larval mortality and deformities, as well as slightly elevated embryo mortality. Larvae exposed to bitumen deposits and refinery sediments were significantly smaller in body size (shorter) than controls. Exposure to reference sediments and controls showed negligible embryo mortality and excellent larval survival. Sediment analyses using GC-MS demonstrated high levels of C_{1-4} PAH compounds. ELS sediment toxicity tests may prove to be valuable tools in the monitoring and assessment of petroleum pollution.

Definition of Sediment Toxicity Zones around Oil Development Sites: Dose Response Relationships for the Monitoring Surrogates Microtox®, Amphipods and Polychaetes Exposed to Hibernia Source Cuttings Containing a Synthetic Base Oil. C. Andrews[1], J.F. Payne[1], S.A. Whiteway[2] and K. Lee[3]. [1]Department of Fisheries and Oceans, St. John's, NF; [2]Jacques Whitford Environment Ltf., St. John's, NF; [3]Department of Fisheries and Oceans, Maurice Lamontage Institute, Mont-Joli, Qc.

Microtox® and amphipod bioassays are commonly employed as monitoring surrogates for sediment toxicity including around oil development sites. Dose-response relationships have been developed for these surrogates in studies with authentic Hibernia source cuttings containing an aliphatic hydrocarbon based drilling fluid. The cuttings demonstrated a similar and very low acute toxicity potential in both the Microtox® and amphipod bioassays. Distinct toxicity was demonstrated with sediments containing approximately 6,000 mg/kg of diesel range hydrocarbons. Diesel range hydrocarbons are also being measured in sediments in monitoring programs in the offshore. This permitted extrapolations to be carried out on the potential size of toxic zones for the monitoring surrogates stemming from disposal of drill cuttings enriched in the drilling fluid in question. The first extrapolation was carried out on the basis of constant hydrocarbon input over time in relation to reference concentrations found at various distances from the rig site after 2 years of development. The other extrapolation was carried out assuming 36% of the wells have already been drilled. Both extrapolations indicated little or no risk for toxicity to sediment monitoring surrogates even as close as 1,000 m or less from the rig site over the life of the project. Risks could also be further reduced at development sites with greater contaminant dispersion potential (Supported by Program on Energy Research and Development).

The Fate of Butyltin Compounds in Nordic Coastal Ecosystems: A Mass Balance Model Development Applied to the Saguenay Fjord, Canada. L. Viglino and É. Pelletier. Institut des sciences de la Mer de Rimouski (ISMER), Université du Québec à Rimouski, Rimouski, Qc.

Tributyltin (TBT) is considered as the most toxic chemical that had ever been deliberately introduced into the aquatic environment. TBT is still used as antifouling agents in marine paints for large commercial vessels. It was responsible for major slump in stock of commercial molluscs and the disruption of the structure of gastropod populations. Even 10 years after its regulation by most industrial countries, TBT remains a major threat for estuarine and coastal environments. To estimate impacts and ecology risks of organotins is getting a real crucial issue, particularly for sediments and biota living in the vicinity of navigation channels and dredging dumps areas.

The objective of this work is to develop a global environmental model of TBT and its metabolites (MBT, DBT) in nordic coastal ecosystems and to validate and apply this model to the Saguenay fjord. This study will be based on assessment flows and a mass balance establishment between different boxes (dissolved particulate matter and biota) in the coastal system. It includes mechanism studies controlling butyltins (TBT, DBT, MBT) reactivity, specific chemistry, transformation and transport in coastal ecosystem. The first work consists in building a conceptual model describing relationships existing between the main compartments of a coastal marine ecosystem and principal mechanisms of the distribution of butyltins. In second step, the

work will be oriented towards a quantitative model predicting the fate of butyltins in this ecosystem and where a series of chemical constants and variables have to be quantified (tributyltin load, freshwater and seawater inflows, partitioning coefficient between sediment and water, concentrations of all butyltin species in water, sediment and biota). The validity of the model will be tested by comparing calculated data with measured environmental concentrations.

Biomonitoring, Bioaccumulation and Biomarkers

Incidence of Deformities in Brook Trout (*Salvelinus fontinalis*) Exposed to Elevated Se Downstream from Coal Mining Activity in Alberta's Northeastern Slopes Region. J. Holm[1], R.E. Evans[2], K. Wautier[2], C.L. Baron[2], P. Siwik[3], G. Sterling[3] and V.P. Palace[2]. [1]Department of Zoology, University of Manitoba, Winnipeg MB; [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; [3]Sustainable Resource Development, Edson, AB.

Selenium is an essential trace element, but at even slightly elevated concentrations can become a potent teratogen in fish. Elevated Se in surface waters downstream from coalmines in Alberta and British Columbia have recently been identified. While the sources of Se are still not well understood, the literature suggests that at current waterborne concentrations, there is the potential for teratogenic effects to occur in resident fish populations. Teratogenesis related to Se arises from uptake of elevated Se from the diet by adult females, deposition of high concentrations of the metalloid into her eggs, and the subsequent substitution of Se for S during the assembly of structural and functional proteins in the rapidly developing offspring. To examine the potential for teratogenesis related to elevated Se, spawn from adult brook trout were collected downstream from coal mining activity as well as from a reference site. Eggs and milt were transported to the laboratory, fertilized and reared to the swim up stage when they were preserved for evaluation of deformities. Tissues from the spawning adults were also dissected for analysis of Se as well as other metals/metalloids. Results indicate increased incidence of craniofacial and yolk sac deformities in offspring of fish collected downstream of coal mining activity.

Trophic Transfer of Retene and Phenanthrene in Boreal Sea Star, *Leptasterias polaris* and Resulting Immune Response. S. Le Breton and É. Pelletier. Institut des Sciences de la Mer de Rimouski (ISMER), Université du Québec à Rimouski, Rimouski, Qc.

The objective of the project was to study the trophic transfer of two light PAHs with related structures, retene (Re) and phenanthrene (Phe), through a single link estuarine food chain using the mussel-sea star model and to observe the effects of bioaccumulated compounds on the immune system of the sea star. Changes in the count, the viability, the neutral red retention and the phagocytic activity of amoebocytes present in the coelomic fluid were determined using microplate techniques. This study was conducted with boreal sea star, *Leptasterias polaris*, feeding contaminated blue mussels, *Mytilus edulis*, (14.5 mg/kg wet weight tissue) during a 21 d exposure period.

Chemical analysis indicated an important bioaccumulation of both PAHs at a similar level in gonads (Re = 120±95 mg/kg and Phe = 140±106 μg/kg), but retene (613±338 mg/kg) averaged about 4 times phenanthrene concentration (145±104 μg/kg) in pyloric caeca. The average value in coelomic fluid was 3±1 μg/L for retene and 11±5 μg/L for phenanthrene. No significant differences were observed in immuno-assays on amoebocytes between control and contaminated starfish (n=6) indicating no apparent effect of these xenobiotics on the defence system of the sea star. Complementary *in vitro* experiments were conducted by exposing directly the coelomic fluid to a large concentration range of both PAHs (C = 10 to 100 mg/L) at several incubation times (3, 6, 12, and 24 h). Results seem to support the hypothesis of an immune system stimulation by both PAHs as phagocytosis increased with exposure times and contaminant concentrations. These results show the high bioaccumulation of light PAHs in sea stars, but their low vulnerability to the presence of these toxicants.

Accumulation, Elimination, Depuration and Turn Over of Tri(n)butyltin by Green Sea Urchin.
J. Mamelona and É. Pelletier. Institut des Sciences de la Mer de Rimouski, Université du Québec à Rimouski, Rimouski, Qc.

We studied the toxicokinetic of tri(n)butyltin (TBT) using green urchin, *Strongylocentrotus droebachiensis*, as a biological model. Urchins were divided into three size classes: <25 mm in test diameter (TD), 35 to 45 mm TD and >50 mm TD. Each size class was subdivided into two groups of 8 separated individuals. During exposition period of 56 d, every 4 d we supplied on each individual a fragment of macro-alga containing TBT, providing a nominal concentration of about 125 μg Sn/kg of urchin in first group, and 25 μg Sn/kg in the second. At the end of exposition period, animals were allowed to depurate during a period of 32 d. Urchins were monitored individually. The ^{113}Sn-TBT was used to study the kinetic, using the non-destructive γ *in-vivo* counting method.

About 63 to 79% of ingested toxicants are retained by urchins. The elimination rate was determined from exponential decreases of retained toxicants at each dose administration cycle (4 d). The depuration rate was determined with the exponential decreases in depuration period. Results shown that elimination is about five fold weaker than depuration. The biological half live of TBT is 173 to 211 d during the accumulation-elimination period, whereas it is 43 to 65 d in depuration period. Results from this study combined with our data from kinetics of TBT adsorption/desorption by macro-algae and the field level of butyltins on urchins and macro-algae permit us to establish a mass balance model of TBT flux within three compartments: seawater, macro-algae and urchins.

Ablation and Regeneration of Catecholaminergic Neurons in Rainbow Trout (*Oncorhynchus mykiss*) Characterized by Melanophore Control. R.W.J. Ryan[1], J.I. Post[1], M. Solc[1], P.V. Hodson[2] and G.M. Ross[1]. [1]Department of Physiology, Queen's University, Kingston, ON; [2]School of Environmental Studies, Queen's University, Kingston, ON.

Genetic, neurochemical, and environmental factors have been implicated in neurodegenerative disease, and a combination of these factors is likely responsible for disease onset and progression. Environmental toxicants associated with Parkinson's disease include organic compounds, reactive oxygen species, and metal ions. We have demonstrated that Zn^{2+} and Cu^{2+} bind with the neurotrophins NGF and BDNF, producing conformational changes that inhibit binding with receptors.

In vitro studies have demonstrated that removal of neurotrophic support by exposure to Zn^{2+} leads to apoptotic neuronal death. Rodent models of toxicant-induced neurodegeneration are hampered by the high resistance of these animals to many environmental toxicants. Extensive literature on aquatic toxicology and the high homology between human and fish neurotrophins make fish a useful model for investigating environmental toxicants and neurodegeneration. Skin color in salmonids is under catecholaminergic control; pigment containing melanophores aggregate when stimulated, resulting in paling. We demonstrate that lesions to nerves innervating melanophores prevent aggregation and produces dark skin color. The time course of return of skin color corresponds to neuronal regeneration, a neurotrophin-dependent event. We further show that exposure to metal ions changes the time course of skin color return; this is likely the result of metal ion-induced loss of neurotrophin support. Observations from this model system may be useful for predicting the risks associated with environmental toxicants and development of neurodegenerative disorders.

Caged Bivalves as an Environmental Monitoring Tool: A Graphical History. S.M. Salazar and M.H. Salazar. Applied Biomonitoring, Kirkland, WA, USA.

We have advocated the use of caged bivalves as an environmental monitoring tool for many years. Our methodology recently has been accepted as a standard guide by the American Society for Testing and Materials. Environment Canada is currently working on a technical guidance to assist practitioners in the proper conduct of these tests. Valuable information has been lost along the way however, with respect to the art and the science of caged bivalve monitoring. It is important to occasionally reflect on the origins of a particular method to gain a proper appreciation for what was originally intended and the details that are so important to an appropriate test. In this poster, the beginnings of the caged bivalve methodology will be traced as we developed it. Many do not realize that this all began as a laboratory test measuring the rate of byssal thread production in marine mussels. This is where we originated the concept of multiple measurements on the same individuals. The glass crystallizing dishes used to hold individual mussels were the beginning of a compartmentalized cage. Although revolutionary at the time, many have adopted this approach. This poster will graphically summarize our work over the last 25 years to show the development of the compartmentalized cage in various forms, the most important discoveries with respect to the development of the methodology, and a glimpse at the future in terms of future development of this potentially powerful tool.

Chronic Effects on the Marine Bivalve Mollusc *Mya arenaria*. J. Pellerin[1], S. Gauthier-Clerc[1], A. Siah[1] and C. Blaise[2]. [1]ISMER, Université du Québec à Rimouski, Rimouski, Qc; [2]Environnement Canada, Centre Saint-Laurent, Montréal, Qc.

Marine invertebrates such as the soft shell clam, *Mya arenaria*, are of crucial importance in estuarine environments for their role in the community structure, while harvesting sustains the regional economy. However, human consumption of clams can alter human health when harvest comes from contaminated areas. Since clams are filter-feeding organisms, bioaccumulation along the plumes of industrial discharges or marinas has been observed for TBT, Hg, Cd, Zn and Cu. Studying the chronic effects of such mixtures of contaminants on *Mya arenaria* could therefore indicate and/or predict problems in the functioning of the ecosystem and the threat for human health.

Thus, in the Saguenay fjord and the St. Lawrence maritime estuary, we have followed the reproductive status of *Mya arenaria* and have found delayed sexual maturation in sites submitted to mixed contamination. Exposure to estrogenic substances can induce vitellogenin and protein synthesis in clams. Thus, we were able, as a preliminary study to characterize in different contaminated sites, the development stages of sexual products for males and females, especially a vitellogenic sequence for females consistent with increased synthetic activities of the gonad. Moreover, decreased progesterone levels were linked to a delayed sexual maturation in the St. Lawrence estuary. Vitellin-like proteins as well as sexual steroids then appear to be valuable biomarkers in the presence of endocrine disruptors.

Oil Sands Regional Aquatics Monitoring Program. T. Van Meer[1] and M. Lagimodiere[2]. [1]Syncrude Canada Ltd., Fort McMurray, AB; [2]Golder Associàtes, Calgary, AB.

The oil sands regional aquatics monitoring program (RAMP) is a multi-company initiative in the oil sands region of northern Alberta. RAMP began in 1997 and is a long-term multi-stakeholder program. The Steering Committee of RAMP is composed of representatives from industry, provincial and federal governments, local aboriginal groups, and environmental organizations. The technical subcommittee of RAMP has developed the core-monitoring program and continues to update it over time.

River monitoring includes hydrology, water quality, sediment quality, benthic invertebrates, fish populations and fish habitat. Wetlands monitoring includes water quality and vegetation including plant community mapping and plant. A monitoring program for acid sensitive lakes and ponds has also been initiated. The focus is on rivers and wetlands in the vicinity of current and proposed oil sands developments. This includes the Athabasca River and several of its major tributaries such as the Steepbank, Muskeg, McKay, Tars, Dover, and Firebag rivers. Rivers south of Fort McMurray such as the Clearwater and Christina rivers have recently been added. The Peace-Athabasca Delta area has also included in the monitoring program.

The results of RAMP are used for many purposes including: detecting and assessing cumulative effects and regional trends, compiling historical and baseline data to characterise variability in the oil sands region, comparing with predictions of environmental impact assessments and to satisfy the monitoring required by regulatory approvals of oil sands development. RAMP also communicates its findings to communities and other stakeholders in the region.

Development of Tools for Rapidly Screening Sub-lethal Stress in Aquatic Organisms: Pilot Studies with the API ZYM[R] Enzyme System. A. Mathieu[1], B. French[1], C. Andrews[2] and J.F. Payne[2]. [1]OCEANS Ltd., St. John's, NF; [2]Department of Fisheries and Oceans, St. John's, NF.

API ZYM[R] provides a semi-quantitative method for rapidly screening a number of enzyme activities using very small samples. The system consists of a strip with 20 microwells containing enzyme substrates and buffer for assaying various hydrolases including phosphatases, esterases, aminopeptidases and glycosidases. The metabolic by-products produced during the incubation period are detected via colored reactions which can be readily assessed with a Reading Table. The system has been mostly used to date in studies with bacteria and fungi to identify groups or strains of organisms upon culturing under various conditions.

Pilot studies have been carried out with the API ZYM[R] system in relation to: [1] enzyme changes in lobster caged in St. John's Harbor, [2] assays of samples of raw water from St. John's Harbor, [3] secretion of mucus containing enzyemes in fish exposed to hypoxic stress, [4] enzyme differences in the spleen of fish exposed to petroleum hydrocarbons, and [5] differential inhibition of brain enzymes in exposure *in vitro* with Hg. These pilot studies have shown that the technique has potential for rapid screening tissue homogenates and biological fluids from different species of aquatic organisms as well as directly examining raw waters associated with sewage contamination. The technique also has potential for investigating mechanisms of chemical toxicity. (DFO contribution supported in part by the Environmental Studies Strategic Fund.)

PAH Bioaccumulation and Export by Dipterans in Wetlands of the Alberta Oil Sands. K.D. Ganshorn and J.J.H. Ciborowski. Department of Biological Sciences, University of Windsor, Windsor, ON.

We assessed bioaccumulation and export of PAHs to the terrestrial environment via dipterans inhabiting wetlands of the Athabasca oil sands. The mining and extraction of bitumen from the oil sands generates oil sands process water (OSPW), which must be stored and detoxified prior to release. The viability of using wetlands for detoxification is being considered. Adult dipterans were collected using UV light traps at a reference wetland and an OSPW wetland and body burdens of various classes of PAHs were determined. Biota-sediment bioaccumulation factors (BSAFs) were calculated for each class of PAHs at each wetland. Chironomid (Diptera) productivity estimates were used in conjunction with the PAH burdens in adult dipterans to estimate if dipterans originating from the OSPW wetland export more PAHs than those originating from the reference wetland.

Body burdens of PAHs in emergent Diptera were generally greater at the reference wetland than at the OSPW wetland. Chironomid productivity of the reference wetland was approximately 7 times greater than that of the OSPW wetland. Emergent dipterans from the OSPW wetland had lower BSAFs than dipterans from the reference wetland, suggesting that there may be a factor present in OSPW wetlands causing reduced bioavailability of PAHs. Alternatively, OSPW dipterans may have adaptations that confer a greater ability to metabolize PAHs. Adult dipterans emerging from the reference wetland exported more PAHs than did OSPW-emerging chironomids. This may be due to both greater bioaccumulation and higher rates of secondary production. Nearby OSPW wetlands possibly contributed dipterans to the reference wetland light trap sample thereby inflating the observed BSAF.

Endocrine Disruptors

Fathead Minnow Exposure to Ethinylestradiol from Egg or Juvenile Stage. J.L. Parrott, G.G. Fodor and B.R. Blunt. Environment Canada, National Water Research Institute, Burlington, ON.

The goal of this study was to assess the use of fathead minnow full lifecycle assay for detection of endocrine disrupting substances and to see whether the test could be shortened by shorter exposures (8, 15, 30 or 60 d) or by exposing juvenile ("teenager") fathead minnows, just before onset of secondary sex characteristics, and following these through maturation and reproductive phases. Fathead minnow eggs or "teens" were exposed to 0, 0.32, 1, 3.2, 10, 32 ng/L (nominal) ethinylestradiol (EE2) in a flow-through exposure system. Actual exposure concentrations measured by EE2 radioimmunoassay were 70 to 90% of nominal. Effects on growth, secondary sex characteristics, physiological alterations (changes in liver size, gonad size, fecundity) and reproductive performance (fertilization and hatching success) were examined.

No statistically significant changes were seen in eggs (hatch, mortality) or fry at 8 or 15 d (length, weight, mortality) or juveniles up to 30 d post-hatch. At 60 d, fish were significantly smaller (32 ng/L), and secondary sex characteristic (measured as ovipositor index) was the most sensitive measure of effect in fathead minnows exposed to >3.2 ng EE2/L. Following the fish through maturation, reproduction, and sampling at 150 d provided several endpoints that responded to EE2. Females were larger and had increased liver size. Sex ratio was altered, with the three highest concentrations having all female fish. The most sensitive endpoint was fertilization success (affected at 0.32 ng/L). Exposures of "teen" fish resulted in similar findings, but alterations in maturation and sex characteristics required 10-fold higher EE2 concentrations. Lifecycle tests examining the fertilization success endpoint were more sensitive than partial lifecycle or other endpoints of effect that we examined.

Removal of Toxic Substances and Contaminants of Concern by Municipal Wastewater Treatment. N.D. Domey[1], J. Tigner[1], E. Karalis[1], A. Atkinson[1] and A. Schnell[2]. [1]Environment Canada, Hull, Quebec; [2]Hydromantis, Inc., Cambridge, ON.

The potential effectiveness of conventional biological treatment processes for the removal of toxic substances and other contaminants in municipal wastewaters is of importance to regulatory agencies. Some natural and synthetic hormones have demonstrated endocrine disrupting effects on aquatic organisms but their removal during wastewater treatment has not been well characterized. In this investigation, the removal effectiveness of endocrine disrupting substances, along with alkylphenolics, and other possible toxic substances, from wastewater and wastewater sludge is examined and critical process parameters for maximizing biodegradation are identified. In addition, recommendations from pertinent scientific investigations are cited to address remaining knowledge gaps relevant to wastewater treatment.

Immunomodulatory Effects of Pesticides on Fish Macrophage Function. M. Kohli and N.F. Neumann. Environment Canada, National Water Research Institute, Burlington, ON.

Pesticides such as atrazine, metribuzine, cyanazine, metolachlor and endosulfan have been used in Ontario in quantities greater than 25,000 kg/year for crop protection. Recent evidence suggests that these pesticides may be potential endocrine disruptors. An in-depth review of the environmental toxicity and environmental concentrations of these pesticides is being examined by Environment Canada. In addition to their endocrine disrupting potential, some of these pesticides have been shown to have potent immunomodulatory effects in mammals and fish. We have examined the effect that exposure to these pesticides has on antimicrobial functions of fish macrophages. Macrophages are white blood cells responsible for providing innate cellular immunity against pathogens. Macrophages kill pathogens via several mechanisms, including the production of cytotoxic molecules (i.e., H_2O_2, OH^-, HOCl, NO^-). These pesticides have been tested for their ability to modulate production of reactive oxygen and nitrogen intermediates by fish macrophages.

Effect of 4-nonylphenol and Estrogen on Plasma IGF-1 of Atlantic Salmon Smolts. J.T. Arsenault[1,2], W.L. Fairchild[1], D.L. MacLatchy[2], K. Haya[3], L.E. Burridge[3], D.T. Bennie[4] and S.B. Brown[4]. [1]Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB; [2]University of New Brunswick, Saint John, NB; [3]Department of Fisheries and Oceans, Biological Station, St. Andrews, NB; [4]Environment Canada, National Water Research Institute, Burlington, ON.

It has been hypothesized that 4-nonylphenol (4-NP) is linked to declines in Atlantic salmon populations, with effects being related to the smolt stage. In 1998, a study verified that 4-NP can affect parr-smolt transformation (PST) of Atlantic salmon such that subsequent growth in sea water is impaired. The mechanism by which 4-NP affects growth in smolts is not known. Growth hormone (GH) and Insulin-like Growth Factor-1 (IGF-1) are key hormones that modulate PST and growth. To determine if the effect of 4-NP on growth is a result of a disruption in the GH/IGF-1 axis, a follow-up study was conducted. Smolts were exposed to environmentally-relevant pulse doses of water-borne 4-NP (20 ng/L) and to sustained doses of estradiol (100 ng/L) as a positive control. Treatments occurred at three time windows within the final stages of PST. Subsequent growth in sea water and plasma IGF-1 were evaluated.

Preliminary results show that plasma IGF-1 patterns of smolts treated with 4-NP are significantly different from controls. Results suggest that 4-NP's mechanism of action may involve disruption in the GH/IGF-1 axis. If the effects exerted by 4-NP are due to its estrogenic potential, then estrogenic activity stemming from other sources might influence present-day salmon populations.

Endocrine Activity in Municipal Sewage Treatment Plant Effluents and Activated Sludge Extracts. B.K. Burnison[1], T. Neheli[1], D. Nuttley[1], H.-B. Lee[1], T. Peart[1], M.R. Servos[1], G.J. Van Der Kraak[2], A. Hobby[2], R. McInnis[2] and A. Jurkovic[1]. [1]National Water Research Institute, Environment Canada, Burlington, ON; [2]Department of Zoology, University of Guelph, Guelph, ON.

A variety of estrogenic responses such as intersex and induction of vitellogenin have been identified in fish exposed to municipal effluents in Europe, the US and Canada. Estrogenic compounds have been identified in municipal sewage treatment plant effluents and included industrial chemicals (alkylphenols and bisphenol-A) and natural/synthetic estrogens (17β-estradiol, estrone and 17-α-ethinyl estradiol). These compounds were also found in the STP effluents in this study and at similar concentrations as reported in other countries. High-pressure liquid chromatography (HPLC) was used to identify estrogenic compounds present in various southern Ontario STP effluents using the yeast estrogen screen (YES) as the biological end point. An unknown estrogenic substance was found in one STP effluent that was relatively polar. The identity of this substance is still under investigation. Water extracts from activated sludge did not contain significant estrogenicity while methanol extracts had high YES activity, caused by the presence of the hydrophobic alkylphenolic compounds. The presence of androgenic substances was determined using an androgen receptor-binding assay.

Androgenic Effects of Tributyltin in Three Marine Gastropods. D. Sotornik and L.E.J. Lee. Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Tributyltin (TBT) is an antifouling component in marine vessel paints that has been shown to cause severe endocrine disturbances in many organisms at very low concentrations. Regulations for the environmental input of TBT have been in place for several years, however, TBT is still in use in large vessels and its effects on the overall well being of various organisms are now being questioned. Imposex is a condition observed in several marine snails in which male characteristics become imposed over female structures as a result of TBT exposure. This has led to severe gastropod populations declining at various global sites. Three marine snails abundant in Canadian eastern shores, *Nucella lapillus, Littorina littorea* and *Ilyanassa obsoleta,* were evaluated for TBT effects on imposex and other physiological parameters including heart rate and immunological impairment as measured by hemocyte phagocytic assays. The mechanisms of action of TBT *in vivo* and *in vitro*, at the organismal and cellular levels will be presented. (Supported by PetroCanada Young Innovator Award)

Developmental, Behavioural, and Reproductive Effects Experienced by Japanese Medaka (*Oryzias latipes*) in Response to Short-term Exposure to an Organochlorine Pesticide. K.L. Gormley and K. L. Teather. Department of Biology, University of Prince Edward Island, Charlottetown, PE.

Japanese medaka *(Oryzias latipes*) were exposed to concentrations of 0.01, 0.10, and 1.0 µg/L of the pesticide endosulfan for 24 h beginning either 4 to 6 h post-fertilization or 4 to 6 h post-hatch to determine effects on hatching time, growth, mobility, foraging ability, and reproduction.

Eggs exposed to endosulfan took longer to hatch, and the resulting fry were smaller at one week of age and had decreased mobility at two weeks of age. Upon reaching sexual maturity, these individuals also produced fewer eggs, and these eggs took significantly longer to hatch. Medaka exposed to endosulfan shortly after hatching did not differ in early mobility or foraging ability; however, upon reaching sexual maturity, these individuals also produced fewer eggs. The dose-response curve for many of the tests was not linear, with medaka exposed to intermediate concentrations of endosulfan (0.10 µg/L) exhibiting the greatest response.

Evaluating the Assessment Endpoints for Alkylphenols in the Canadian Environment: Addressing the Endocrine Issue. È.B. Dussault, E. J. Murphy, R. McInnis, B.K. Burnison, M.E. McMaster, H.-B. Lee, J.P. Sherry, D.T. Bennie and M.R. Servos. Environment Canada, National Water Research Institute, Burlington, ON.

Nonylphenol polyethoxylates are widespread chemicals used as nonionic surfactants and discharged in water sewage treatment plants. Although they have relatively low toxicity levels, the toxicity of their degradation products, nonylphenol (NP), nonylphenol ethoxylate (NPEO) and nonylphenol ethoxycarboxylate (NPEC), is significantly higher. In addition, they are found in the environment at concentrations that may be harmful to aquatic organisms. The potential of these substances to disrupt endocrine function has been reported, but is still under debate. While some studies have shown the estrogenicity of NPEO and NPEC to be slightly lower than NP, others have shown much lower levels of estrogenicity for the NPEC, if any; this could have serious implications, given its relative abundance in the environment. Consequently, studies were undertaken to investigate the relative estrogenicity of these chemicals to juvenile rainbow trout (*Oncorhynchus mykiss*).

Fate of Environmental Estrogens in Hog Manure Applied to Agricultural Fields in Southwestern Ontario. M.R. Servos[1], R. McInnis[1], A. Jurkovic[1], K. Terry[1], B.K. Burnison[1], B.-H. Lee[1], G.J. Van Der Kraak[2], and E. Topp[3]. [1]Environment Canada, National Water Research Institute, Environment Canada, Burlington, ON; [2]Department of Zoology, University of Guelph, Guelph, ON; [3]Southern Crop Protection and Food Research Centre, Agriculture Agri-Food Canada, London, ON.

A number of estrogenic compounds, including 17β-estradiol, estrone and the phytoestrogen equol, have been identified in animal manures. To address if animal wastes are capable of entering waterways and exposing fish to estrogenic compounds a detailed study was conducted during normal manure applications at a farm site in southwestern Ontario. The manure from approximately 1,000 sows was stored over the winter in open tanks and then spread in the spring immediately prior to planting. The site has sandy soils, with tile drainage. Samples of manure, soil, tile drainage and stream water were taken after the application of approximately 6,000 gal/acre of manure to fields from which tile drainage enter the stream directly. Samples were collected immediately after the manure applications as well as after major rainfall events. Samples were examined specifically for 17β-estradiol, estrone and equol and extracts were also assayed for estrogenic activity using the YES assay.

The equol, was found in high concentrations in tile and river water immediately after application and also declined rapidly. Only during the initial peak, immediately after application, were natural estrogens 17β-estriadiol and estrone detectable in tile water. Estrogenicity in tile drains corresponded to the peak in the equol concentration (up to 265 ng/L) immediately after application and after the first rainfall. The pattern of estrogenic responses and equol in the tile and stream water parallel the pattern seen for fecal coliforms and ammonium measured in the same samples. The subtle differences in tiles and stream water is consistent with that expected based on the water quality and knowledge of spreading pattern and timing. The results suggest that estrogenic compounds can reach adjacent streams. The significance of this low level, intermittent exposure to stream biota is not yet known.

Contaminant Dynamics in Limnological Systems

Selective Application of Biocides to Eradicate Invasive Aquatic Species. A.J. Niimi. Department of Fisheries and Oceans, Burlington, ON.

Biocides like rotenone have been used as a fisheries management tool to control or eradicate undesirable fish species since the 1920's. Copper-based compounds have been used on some invertebrate species, and 2-4-Dichlorophenoxyacetic acid-based compounds on aquatic plants. Biocides tend to be nonselective in the species affected, but is one of the most effective means of eradicating a species recognizing the difficulties of working in the aquatic environment. Selective use of biocides is being considered to deal with accidentally introduced exotic species. Primary focus would be on newly introduced exotics that may become an invasive species which can cause major ecological and economic problems. The effects of an invasive species have been well documented in large systems like the Great Lakes where biological communities and trophodynamics were severely altered. Treatment may be successful in areas where colonization is localized, geomorphology of the area or artificial barriers could contain the biocide, and the decision to eradicate is made rapidly because that option may no longer be possible once a species has extended its range. There are many technical problems and ethical issues that must be addressed; nevertheless this approach appears to the only method currently available that may effectively deal with a newly reported invasive species.

Elevated Mercury Concentrations in Remote Lake Albert (Uganda, East Africa). L.M. Campbell[1], R. Muggide[2], S.B. Wandera[2], R.E. Hecky[1] and D.G. Dixon[1]. [1]Department of Biology, University of Waterloo, Waterloo, On; [2]Fisheries Resources Research Institute, Jinja, Uganda.

Mercury (THg) in the African Great Lakes is poorly studied despite the high economic and dietary value of the freshwater fisheries to regional economies and local people. Recent studies from Lakes Malawi, Victoria and other African lakes have suggested that THg concentrations in fish rarely exceed WHO guidelines for at-risk groups (200 µg/kg). As a result, most authors have suggested that mercury from fish is not a serious human health concern. However, Lake Albert, a remote western rift valley lake in northern Uganda and D. R. Congo, has high THg concentrations in fish and water. Predator fish in Lake Albert consistently reach concentrations exceeding 1,000 µg/kg. Lake Albert water concentrations are above 2 ng/L and have reached nearly 10 ng/L in deeper waters. Stable nitrogen and carbon isotopes are used to characterize the food web interactions in Lake Albert and to estimate the bioaccumulation factors (BAFs). The BAF value and potential sources of mercury are compared to these for Lakes Victoria and Malawi. Water column profiles are compared to Lake Victoria and other lakes. We also discuss the potential sources of THg (which may be of natural origins) and the potential human health impacts from elevated THg in fish from Lake Albert.

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BEST STUDENT PAPER AWARDS/ PRIX POUR LES MEILLEURS EXPOSÉS PAR DES ÉTUDIANTS

BEST PLATFORM PAPER AWARD

S.J. Crocquet de Rosemond
Toxicology Centre, University of Saskatchewan, Saskatoon, SK.

The Use of TIEs to Characterize Toxic Constituents in a Diamond Mine Effluent.

BEST POSTER PAPER AWARD

J. Muscatello.
Toxicology Centre, University of Saskatchewan, Saskatoon, SK.

Toxicity of Uranium Over the Life Cycle of the Aquatic Invertebrate *Chironomous tentans*.

2nd Place Platform Presentation
A.E. Winchester. Department of Biology, Queen's University, Kingston, ON.

3rd Place Platform Presentation
A.J. Bartlett. Department of Biology, University of Waterloo, Waterloo, ON.

2nd Place Poster Presentation
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3rd Place Poster Presentation
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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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