Proceedings of the 26th Annual Aquatic Toxicity Workshop: October 4-6, 1999, Edmonton, Alberta

Comptes rendus du 26e atelier annuel sur la toxicité aquatique: du 4 au 6 octobre 1999, Edmonton, Alberta

Editors/Éditeurs

E.G. Baddaloo[1], M.H. Mah-Paulson[2], A.G. Verbeek[3] and A.J. Niimi[4].

[1]Government of Nunavut, Sustainable Development, Iqaluit, NU, X0A 0H0;
[2]O'Connor Associates Environmental Inc., Calgary, AB, T2P 0M9;
[3]Conor Pacific Environmental Technologies Inc., Edmonton, AB, T6E 6A8;
[4]Department of Fisheries and Oceans, Bayfield Institute, Burlington, ON, L7R 4A6.

1999

Canadian Technical Report of Fisheries and Aquatic Sciences 2293

Rapport technique canadien des sciences halieutiques et aquatiques 2293

Fisheries and Oceans

Pêches et Océans

Canada

Canadian Technical Report of Fisheries and Aquatic Sciences

Technical reports contain scientific and technical information that contributes to existing knowledge but which is not normally appropriate for primary literature. Technical reports are directed primarily toward a worldwide audience and have an international distribution. No restriction is placed on subject matter and the series reflects the broad interests and policies of the Department of Fisheries and Oceans, namely, fisheries and aquatic sciences.

Technical reports may be cited as full publications. The correct citation appears above the abstract of each report. Each report is abstracted in *Aquatic Sciences and Fisheries Abstracts* and indexed in the Department's annual index to scientific and technical publications.

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

Technical reports are produced regionally but are numbered nationally. Request for individual reports will be filled by the issuing establishment listed on the front cover and title page. Out-of-stock reports will be supplied for a fee by commercial agents.

Rapport technique canadien des sciences halieutiques et aquatiques

Les rapports techniques contiennent des renseignements scientifiques et techniques qui constituent une contribution aux connaissances actuelles, mais qui ne sont pas normalement apropriés pour la publication dans un journal scientifique. Les rapports techniques sont destinés essentiellement à un public international et ils sont distribués à cet échelon. Il n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques du ministère de Pêches et des Océans, c'est-à-dire les sciences halieutiques et aquatiques.

Les rapports techniques peuvent être cités comme des publications complètes. Le titre exact paraît au-dessus du résumé de chaque rapport. Les rapports techniques sont résumé dans la revue *Résumé des scieces aquatiques et halieutiques*, et ils sont classés dans l'index annuel des publications scientifiques et techniques du Ministère.

Les numéros 1 à 456 de cette série ont été publiés à titre de rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457 à 714 sont parus à titre de rapports techniques de la Direction générale de la recherche et du développement, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715 à 924 ont été publiés à titre de rapports techniques du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom actuel de la série a été établi lors de la parution du numéro 925.

Les rapports techniques sont produits à l'échelon régional, mais numérotés à l'échelon naitonal. Les demandes de rapports seront satisfaites par l'établissement auteur dont le nom figure sur la couverture et la page du titre. Les rapports épuisés seront fournis contre rétribution par des agents commerciaux.

Canadian Technical Report of
Fisheries and Aquatic Sciences

Rapport technique canadien des
sciences halieutiques et aquatiques

Canadian Technical Report of Fisheries and Aquatic Sciences 2293/
Rapport technique canadien des sciences halieutiques et aquatiques 2293

1999

Proceedings of the 26th Annual Aquatic Toxicity Workshop: October 4-6, 1999, Edmonton, Alberta / Comptes rendus du 26e atelier annuel sur la toxicité aquatique: du 4 au 6 octobre 1999, Edmonton, Alberta

Editors/Éditeurs

E.G. Baddaloo[1], M.H. Mah-Paulson[2], A.G. Verbeek[3] and A.J. Niimi[4].

[1]Government of Nunavut, Sustainable Development, Iqaluit, NU, X0A 0H0; [2]O'Connor Associates Environmental Inc., Calgary, AB, T2P 0M9; [3]Conor Pacific Environmental Technologies Inc., Edmonton, AB, T6E 6A8; [4]Department of Fisheries and Oceans, Bayfield Institute, Burlington, ON, L7R 4A6.

Cat. No. Fs 97-6/2293
ISSN 0706-6457

Correct citation for this publication/On devra citer la publication comme suit:

Baddaloo, E.G., M.H. Mah-Paulson, A.G. Verbeek and A.J. Niimi (eds). 1999. Proceedings of the 26th Annual Aquatic Toxicity Workshop: October 4-6, 1999, Edmonton, Alberta. Can. Tech. Rep. Fish. Aquat. Sci. 2293: 155 p. / Baddaloo, E.G., M.H. Mah-Paulson, A.G. Verbeek and A.J. Niimi (eds). 1999. Comptes rendus du 26e atelier annuel sur la toxicité aquatique: du octobre 4 au octobre 6 1999, Edmonton, Alberta. Rapp. tech. can. sci. halieut. aquat. 2293: 155 p.

PREFACE/PRÉFACE

The 26th Annual Aquatic Toxicity Workshop was held at the Sheraton Grande Hotel in Edmonton, Alberta, October 4 to 6, 1999. The Workshop included 8 plenary presentations, 101 platform and 50 poster papers. Total attendance was 316.

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 26e atelier annuel sur la toxicité a eu lieu L'Hôtel Sheraton Grande, Edmonton, Alberta les 4 au 6 octobre 1999. Le atelier a donné lieu a 8 communications lors de séances plénières, 101 exposés d'invités d'honneur 50 communications par affichage. 316 personnes ont assisté au atelier.

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

EDITORS COMMENTS/REMARQUES DES ÉDITEURS

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.

ORGANIZING COMMITTEE/COMITÉ D'ORGANISATION

Organizing Committee

Earle G. Baddaloo, Organizing Chair. Government of Nunavut, Iqaluit
Detlef (Deib) Birkholz. Enviro-Test Laboratories, Edmonton
Maureen A. Brown. Maxxam Analytics Inc., Edmonton
Melinda Clendenan. EBA Engineering Consultants Ltd., Edmonton
J. Stephen Goudey. HydroQual Laboratories Ltd., Calgary
May Mah-Paulson. O'Connor Associates Environmental Inc., Calgary
Allen G. Verbeek. Conor Pacific Environmental Technologies Inc., Edmonton

Technical Committee

Detlef (Deib) Birkholz. Enviro-Test Laboratories, Edmonton
Maureen A. Brown. Maxxam Analytics Inc., Edmonton
Melinda Clendenan. EBA Engineering Consultants Ltd., Edmonton
Margaret Fairbairn. Environment Canada, Edmonton
J. Stephen Goudey. HydroQual Laboratories Ltd., Calgary
May Mah-Paulson. O'Connor Associates Environmental Inc., Calgary
Allen G. Verbeek. Conor Pacific Environmental Technologies Inc., Edmonton

SPONSORS/COMMANDITAIRES

Major Supporting Sponsors

Alberta Environment, Edmonton, AB
Department of Fisheries and Oceans, Central and Arctic Region
Environment Canada, Prairie and Northern Region
Enviro-Test Laboratories, Edmonton, AB
Government of Nunavut, Sustainable Development
O'Connor Associates Environmental Inc., Calgary, AB

Major Sponsors

Conor Pacific Environmental Technologies Inc., Edmonton, AB
EBA Engineering Consultants Ltd., Edmonton, AB
HydroQual Laboratories Ltd., Calgary, AB
Maxxam Analytics, Edmonton, AB
Millar Western Forest Products Ltd., Whitecourt, AB
Northwest Territories Power Corporation, Hay River, NT
Government of Northwest Territories, Resources, Wildlife and Economic Development
Weyerhaeuser Canada, Grande Prairie, AB

Sponsors

Alberta Newsprint Co., Whitecourt, AB
Azur Environmental, Carlsbad, CA
BC Research, Vancouver, BC
Beak International Inc., Brampton, ON
Bovar Waste Management, Calgary, AB
Canadian Association of Environmental Analytical Laboratories
Canadian Council of Ministers of the Environment
Cantox Environmental Inc., Calgary, AB
Cogema Resources Inc., Saskatoon, SK
ERM Environmental Consulting, Vancouver, BC
ESG International Ltd., Guelph, ON
EVS Environment Consultants, North Vancouver, BC
G.R. Craig & Associates, Bolton, ON
G3 Consulting Ltd., Richmond, BC
Golder Associates Ltd., Calgary, AB
Government of Alberta, Treasury Branch, Edmonton, AB
Hudson Bay Mining and Smelting Co., Flin Flon, MB
Jacques Whitford Environment Ltd., St. John's, NF
Komex International Ltd., Calgary, AB
National Research Council of Canada
Nova Chemicals, Red Deer, AB
Royal Bank of Canada, Edmonton, AB
Stantec Consulting Ltd., Calgary, AB

TABLE OF CONTENTS/TABLE DES MATIÉRES

PLENARY SESSION/SÉANCE PLÉNIÈRE

Appropriate Uses of Environmental Quality Guidelines - Issues and Solutions

Environmental Quality Guidelines Provide Guidance Not Goals. P.M. Chapman. EVS Environment Consultants, North Vancouver, BC.

Environmental quality values (EQVs), which may be referred to as "guidelines," "criteria" or "standards" depending on jurisdiction and usage, are too often considered "bright lines" demarcating the difference between protecting as opposed to abusing the environment and/or human health. However, EQVs for the same substances vary between geographic areas. In the case of U.S. and Canadian chronic water quality ecological criteria/guidelines, most substance-specific EQVs differ by >2x. In some cases the difference exceeds an order of magnitude (e.g., for freshwater: As, Cd, pentachlorophenol, chlorpyrifos). Yet the Canada-U.S. border is clearly not of ecological significance. Similar differences can also be found between U.S. states and Canadian provinces and even between government agencies. Comparing the new (August 1999) ANZECC water quality values to U.S. and Canadian water quality values, factor differences >2 exits for 86% of freshwater substances and for 81% of saltwater substances, Even greater differences exist in the case of sediment quality values for North America and abroad. Some EQVs, for instance total petroleum hydrocarbon (TPH) measurements, can be misleading surrogates for complex mixtures of substances. EQVs, though initially based on science, are ultimately promulgated based on policy, not science. Thus they cannot be regarded as goals but rather as guidance. They should only be used as the first, screening stage (problem formulation) in a ecological risk assessment framework to identify substances of potential concern for further investigation.

Canadian Environmental Quality Guidelines: Promoting a Nationally Consistent Level of Environmental Quality. R.A. Kent, C.L. Gaudet, P-Y. Caux and S.L. Smith. Environment Canada, Guidelines and Standards Division, Hull, QC.

Ambient environmental quality guidelines (EQGs), criteria, standards are an element of most environmental management regimes worldwide. In Canada, EQGs are recommended benchmarks for the quality of atmospheric, aquatic, and terrestrial ecosystems, and are recognized internationally as a model of cooperative, harmonized action across federal, provincial and territorial environment and health ministries. Canadian EQGs provide the nationally-consistent, science-based targets towards for achieving the highest level of environmental quality for all Canadians the principal vision under the Canada-Wide Accord on Environmental Harmonization. As with most environmental management tools, guidelines are subject to criticism, the majority of which, in our experience, is related to their application outside of a consistent national implementation framework based on sound science as well as technological and socio-economic principles. Variability amongst guidelines will continue as this is primarily a function of their underlying policies or legislation specific to the jurisdiction. Scientific and technical advancements in their derivation continue to be made. Policies and available resources also dictate (to a large extent) their adoption into practise. The success of the Canadian approach therefore is not solely hinged on the guideline development, but on implementation that promotes nationally-consistent level of environmental protection and quality. This model includes providing the most scientifically defensible generic guidelines at a national level for authorized jurisdictions to adopt and/or modify to account for site-specificity.

Development and Application of National Soil Quality Guidelines. T. Nason. Alberta Environment, Environmental Sciences Division, Edmonton, AB.

Soil quality guidelines based on the CCME 1996 protocol are based on defined uses of land - agricultural, residential, commercial and industrial. For each of these, an explicit exposure scenario lays out assumptions concerning receptors to be protected and the level of protection afforded to each. The level of protection targeted is reflected in the toxicological endpoint(s) chosen and the exposure frequency, duration and intensity assumed. Many assumptions are required concerning the fate and behavior of a contaminant in order to estimate exposure for organisms that do not reside continuously in soil (e.g., grazing herbivores, predators, humans). Exposure routes for such receptors are influenced by site features including: depth of contamination, soil texture and organic carbon content, and groundwater properties. Such factors can be taken into account in the application of guidelines as site-specific objectives. Technically, risk-based guidelines could be "recalculated" for nearly any site-specific condition that is explicitly represented in the development protocol (e.g., receptor suites, exposure period, toxicological endpoint, fate and transport models used etc.). In practice, regulators distinguish between "calibration adjustments" that do not alter the management goals reflected in the exposure scenario, and management adjustments that materially change management goals or require on-going risk management. In the latter case, engineered or institutional controls are often required as part of the risk management plan. While most jurisdictions do make use of the latter option for the most difficult sites, there is presently little reliance on guidelines for achieving an appropriate management strategy; rather, site-specific risk assessment is the tool of choice.

Of What Use are Guidelines to Industry? J. Witteman, BHP Diamonds Inc., Yellowknife, NT.

Guidelines serve as a useful reference for industry. They provide topical information on select parameters in different media. However, when regulatory agencies take these same guidelines and interpret them as enforceable standards, this is a matter of concern to industry. In the Lac de Gras area of the Slave Geological Province, water bodies are poorly buffered, pH is commonly less than 6.5 and Al values are commonly greater than the water quality guideline of 5 µg/L. Similarly, sediments are enriched in several trace metals, particularly As, Co, Cu and Zn. In many instances sediment quality guidelines are exceeded. Extensive biological work on these waters and sediments do not indicate any obvious negative effects on the biota. The results certainly do not invalidate water and sediment guidelines but they do point to the difficulty in applying guidelines as enforceable standards. Guidelines should remain guidelines while enforceable standards should be developed based on a risk assessment of the area affected by industry.

When does Regulatory Interpretation become Legal Advice? N.C. Skinner. Conor Pacific Environmental Technologies Inc., Edmonton, AB.

Environmental law is grounded in science, but is driven by the same political and socioeconomic factors that influence all legal regimes. A strict interpretation by an environmental scientist or other professional will be scientifically and rationally sound, but may overreach, or understate, the actual legal requirement. Likewise, a strict legal interpretation of an informal guideline may fail to recognize the actual use and effect that the regulators, and the regulated, place on certain publications, whether lawfully necessary or not. Canadian regulatory frameworks are less rule-oriented, arguably more flexible, inherently more discretionary, and less dependant on mandatory public approval than in the U.S. In addition, the Provinces generally control environmental matters within their own territories, meaning there are a dozen sets of rules. And, most Canadian environmental statutes have limited, if any, judicial review provisions. In Canada informal (i.e., non-legislated) guidelines may be relatively "easy" to work with, on the surface. However, when informal rules are used by officials with broad, and to a great extent ambiguous discretionary powers (as in many Canadian statutory regimes) to service possibly unstated or unclear policy objectives, the resulting administration of many environmental quality guidelines may become difficult, if not impossible to predict. This confusion is exacerbated when a presumption is made that the purpose of a given set of guidelines is its (obvious?) foundation in environmental quality or other scientific base. Science is often indeed necessary to explain "why" some result should be achieved but cannot, itself, answer the question of whether we must achieve that result, or may disregard or avoid it, as a legal matter.

Environmental Quality Guidelines: Do They Really Protect Ecosystems? S.M. Swanson. Golder Associates Ltd., Calgary, AB.

The existence of environmental quality guidelines has led to a "bottom-up" approach to ecological risk assessment that may or may not truly protect ecosystems. Guidelines are developed for individual chemical contaminants. Therefore, the natural tendency is to measure concentrations of these contaminants in various media and then compare these concentrations to guidelines to "screen" for those contaminants that exceed the guidelines. If they do exceed guidelines, then it is assumed that each contaminant must be assessed for the risk it poses to selected ecological receptors. If the risk (usually estimated by a simplistic calculation of the ratio of exposure:effects benchmark) exceeds an arbitrary level (often 1) then it is assumed that some action is required to reduce the risk to the ecosystem. This bottom-up, contaminant-by-contaminant approach ignores: how contaminants may act together; non-chemical stressors; the effects of confounding and mitigating factors in natural ecosystems; the importance of communities of receptors, not just individual populations of selected receptors; adaptation and succession in response to stressors effects on ecosystem function; and natural variation in ecosystem response to stress. Hence, reliance on guidelines can produce ecological risk assessments that are wildly inaccurate and either vastly over-protective or significantly under-protective. As a consequence, the true "net benefit" of dollars spent on remediation and mitigation is usually unknown. This can lead to millions of dollars spent on reducing chemical concentrations to femtogram quantities in the receiving environment while ignoring the fact that the ecosystem is being severely affected by factors such as sedimentation, over-fishing or remediation. The solution to this problem may be to combine the use of guidelines with an ecosystem-based approach. This will often mean starting in the field, rather than the laboratory. Some real-world examples will be presented to illustrate these points.

Environmental Quality Guidelines: Do they Really Protect Human Health? B. Rogers. Toxcon Health Sciences Research Centre Inc., Edmonton, AB.

One of the main objectives of the promulgation of Canadian ambient Environmental Quality Guidelines (EQGs), apart from harmonization for the purposes of a consistent approach across various government jurisdictions, is to set in place generic, scientifically-defensible numerical values that will be protective of the environment as well as of ecological and human receptors. Prior to the implementation of this approach, these EQGs were often generated with varying degrees of scientific rigor resulting in values that could be highly variable between jurisdictions. From the human health perspective, the question became not only was the value protective, but why was it different from that of another jurisdiction. Ideally, any EQG should protect both normal individuals and sensitive sub-populations from the onset of significant adverse health effects prior to the onset of clinical signs or symptoms. Given that EQGs are used broadly from environmental clean-ups, to commercial land transactions, to environmental risk management as well as to develop new regulatory or economic policies, it would seem logical that the best science go into their development. However, this may not always be the case. This presentation will focus on some of the current toxicological issues facing both those who develop and those who use EQGs for the purpose of protecting human health.

PLATFORM PRESENTATIONS/SÉANCE EXPOSÉS

North of 50 Issues

Positive Benefits of Wetlands for Pollution Control from Highways Construction. D.J. Arsenault. EBA Engineering Consultants Ltd., Kelowna, BC.

Wetlands are natural filters of suspended and dissolved solids. Their role in flow attenuation is well documented and they are used extensively in effluent treatment systems for mines and municipal wastewater. Recently, they have been incorporated into highway designs for both temporary and long-term treatment of storm runoff. A large-scale example is the Island Highway on Vancouver Island, BC. Engineered wetlands can be costly to design and construct and do not always take full advantage of the biological efficiency of shallow wetlands for adsorption, absorption, and breakdown of potentially deleterious substances. Shallow wetlands were developed for a design-build highway project in the southern interior of British Columbia for the Ministry of Transportation and Highways. Construction of these wetlands, prior to highway construction, functioned to protect an important fish-bearing stream draining into Okanagan Lake from damage to fish and fish habitat. However, pollution from highways does not stop once construction is complete. Long-term benefits were realized from the elimination of direct discharge into this stream from highway surface runoff. Economically viable long-term benefits that included community involvement were recognized from wetland development. The author also presents examples of other uses for constructed wetlands and their overall benefit to aquatic ecological health.

Midges (Diptera: Chironomidae) as Indicators of Wetland Viability in the Alberta Oil Sands Region. J.J.H. Ciborowski. M.P. Whelly, C. Leonhardt and D. Laing. Department of Biological Sciences, University of Windsor, Windsor, ON.

To assess the biological integrity of wetlands receiving Oil Sands mine process-affected water (OSPW) we surveyed 33 wetlands northeast of Fort McMurray, AB exhibiting a broad range of environmental features and disturbance history. Ten of these had received OSPW. We measured physico-chemical and environmental attributes of each wetland, and collected sediment, zoobenthic, plankton, and chlorophyll *a* samples. Principal components analysis indicated that these wetlands could be ordinated with respect to gradients of three independent suites of environmental features including pH/size/dissolved oxygen; conductivity; and sediment composition (organic content; median particle size). Cluster analysis identified 4 broad groups of wetlands, which were used to select most appropriate reference sites for comparison with each of 3 focal OSPW-affected wetlands. High conductivity wetlands supported greater density but reduced richness of benthic taxa than low-conductivity wetlands whether or not OSPW was present. To evaluate OPSW wetland ability to support and maintain benthic populations, we monitored chironomid larval morphology, density, and production (weekly benthic cores), adult emergence (Davies emergence traps), flight activity (floating sticky traps), oviposition behaviour (pan traps), and ability of eggs to hatch in OPSW and reference water (laboratory observations). Presence of OSPW did not inhibit adult chironomid flight activity or oviposition. Nor did it prevent egg hatching. However, chironomid density, diversity, and secondary production were possibly reduced by OPSW. Incidences of mouthpart deformities in chironomid larvae collected from

OPSW wetlands in low, and no greater than incidences in paired reference wetlands.

Environmental Health Monitoring on Alberta Oil Sands Wetland and Terrestrial Reclamation Areas Using Upper Trophic Level Birds. J.E.G. Smits[1,2], A. Jeroski[2], R. Carter[1] and M. Wayland[3]. [1]Toxicology Centre, [2]Department of Veterinary Pathology, University of Saskatchewan, Saskatoon, SK; [3]Environment Canada, Saskatoon, SK.

On the extensive Oil Sands mining areas in northern Alberta, restoration issues are being addressed through the establishment of reclaimed terrestrial and wetland habitats. Wetlands created from a combination of natural drainage, dyke seepage and tailings pond runoff from mining sites, as well as revegetated terrestrial grassland and wooded areas are viewed as ecologically viable ways of recovering mined areas for use by native flora and fauna. In these studies, wild birds at or near the top of the food chain provide a model for assessing the ecological sustainability of the reclaimed areas. By examining reproductive, immunological and other physiological parameters in song birds and birds of prey nesting on or near wetland and terrestrial reclamation sites, we have a model for detecting subtle and early contaminant-associated changes in warm-blooded, upper trophic level vertebrates. Resident wild raptors, American kestrels (*Falco sparverius*) (1999 only), and tree swallows (*Tachycineta bicolor*) (1997-1999) were investigated during the breeding season of May through July. This was the first year of study using falcons, and the third year using tree swallows as monitors of ecosystem health, in an area where dietary exposure to environmental contaminants may occur directly through the water or via bioaccumulation in the food web. Productivity variables consisting of clutch size, egg weight, hatching success, nestling viability and growth rate, as well as survival to fledging, reflect parental qualities including behavioural aspects of courtship, egg incubation and tending the young, and also provide measures of the feeding, growth rates and competitive fitness of the offspring.

Two tests of immune function were carried out on the kestrel chicks. The phytohaemagglutinin (PHA) test, which entails subcutaneous injection of the mitogen PHA, is known to stimulate migration, accumulation and a measurable proliferation of circulating T-lymphocytes. The delayed type hypersensitivity test (DTH) measures an integrated aspect of cell mediated immunity, and is dependent upon the interaction of T-lymphocytes, cytokines, and antigen presenting cells (macrophages or B-lymphocytes). Together, these tests allow the evaluation of different levels of the immune response. The PHA skin test measures an early, relatively generic response, while the DTH reaction involves a more complex, highly evolved immune reaction. An *in vivo* PHA test was used in tree swallows in 1997 and 1998. Both PHA and DTH tests were conducted *in vivo* on kestrel chicks produced on the oil sands experimental reclamation sites. The DTH test under development for use in kestrels, entailed vaccinating nestlings at 10 d of age with a nonpathogenic antigen, keyhole limpet hemocyanin (KLH), and measuring their response to an intradermal challenge 12 d later.

In the first two years of research on these experimental reclamation sites, tree swallows were the primary indicator species. A study of hepatic enzyme (EROD) induction, a measure of the detoxification efforts of birds on different experimental sites, revealed that birds on the Syncrude reclamation ponds, as well as the Poplar Creek Reservoir reference site had relatively low EROD induction (low detoxification demands on the liver) whereas tree swallows on the Suncor test wetlands had significantly higher EROD induction than reference sites. This increased detoxification effort was not reflected in the T-cell proliferative response to PHA in the tree swallow nestlings, in which there were no differences among sites. However, the birds from the Suncor site laid significantly fewer eggs per clutch than birds at Syncrude, and the Suncor fledglings were smaller than those from the reference site. In 1999, as in previous years, Suncor pairs laid fewer eggs (p=0.009) than those at Syncrude, and the average egg weight was lower at both test sites compared with the reference site (p<0.001).

Immunotoxicology tests in 1999 were conducted only on the kestrel nestlings. The PHA response in nestlings at Syncrude (n=43) was significantly greater than those from Suncor (n=24) (p=0.004), and greater but not significantly so, at Poplar Creek (n=8). The DTH response tended to be strongest at Poplar Creek Reservoir. This apparent contradiction in the T-cell mediated immune response may be an artifact of the low "n" on the reference area during this first year. The weights of kestrel nestlings at 22 d old, were not different among sites. Blood variables evaluated in kestrel chicks at the different sites showed leucocrit and total serum protein concentration were not different, but the hematocrit was significantly lower in the nestlings at the reference site both at 10 d (p=0.009) and 22 d of age (p=0.001). Chicks from the reference had lower mass and hematocrit, which would point towards something possibly interfering with the anabolic processes in these birds. The adult kestrels from one nest were observed to be frequently harassed by a large population of tree swallows nesting in close proximity, which was speculated to have interfered with hunting and certainly would have increased their stress levels. Based upon this pilot study, there were no clear differences in the growth rate of kestrel chicks on the three sites being studied, implying that tailings-associated compounds did not have a limiting effect on the abundance of food available to the adults, nor did it have a noticeable negative effect on feeding behaviour by parents or offspring, nor did it compromise the physiological and biological factors associated with feed utilization and growth rate.

There generally appears to be a muted, but consistent pattern in animals on one of the experimental reclamation sites that suggests subtle, physiological compromise that spans reproductive and immunological function. The EROD induction indicated that birds on the Suncor wetlands required an increased detoxification effort to deal with environmental contaminant exposure. Attracting more kestrels to the reference area in future years will be critical to establish a good reference population that will provide sound baseline data on reproductive and immunological variables. The DTH test, currently being adapted for use in American kestrels in this lab, requires further work to establish the optimal test protocol. Overall, this multifaceted approach to evaluation of environmental health of an industrial site "of concern," using high trophic level avian species has proven feasible, promising and instructive.

Risk Assessment of Contaminated Sites in Northern Environments: Challenges to Conventional Risk Assessment. G.L. Robins, T.L. Knafla and J.H. Sevigny. Komex International Ltd., Calgary, AB.

The use of risk assessment in the evaluation of potential health risks for humans and wildlife can be complicated by variables unique to northern environments. Pertinent variables include the close inter-relationship of first nations peoples with their environment, increased sensitivity to toxicants due to cold stress, and hypothermic response to chemical exposure. The importance of these variables was highlighted while developing a risk management framework and conducting a risk assessment for a large oil and gas facility located in the Northwest Territories. The determination of potential health effects is complicated by the fact that first nations populations are dependent on delicate northern food webs, and thus are sensitive to chemical impacts on their environment. This results in a substantial overlap between human and ecological risk assessments, which are risk concepts that are typically treated separately. The extreme seasonal variation in temperature is another variable of concern. Winter conditions place additional stress on animals and may influence their toxicological response to contaminants. Thus, standard toxicity information used to assess risk to ecological receptors may not be appropriate in cold environments. Hypothermia is induced by several types of chemicals and could be fatal in cold winter months. Risks to populations may be underestimated with conventional risk assessment methods.

Soil Ecotoxicology

Toxicity Assessment of Unrefined Crude Oil Fractions in Soil Ecosystems. J.S. Goudey[1], J.J. Wilson[1,2], A. Chu[2] and J.F. Hatcher[2]. [1]HydroQual Laboratories Ltd., Calgary, AB; [2]Department of Civil Engineering, University of Calgary, Calgary, AB.

Several crude oil contaminated sites have undergone bioremediation to reduce the total extractable hydrocarbons (TEH) in the soil. Most sites cannot reach the current remediation criteria of 1000 mg/kg TEH because of the resistance of the heavier hydrocarbon fractions (i.e., $>C_{25}$) present in unrefined crude oils to biodegradation. To evaluate the contribution of the heavier hydrocarbon fractions to the total crude oil toxicity in soil ecosystems, five fractions obtained by fractional distillation of three different crude oils were tested for potential ecotoxicity after addition to a loam soil. Five fractions of paraffinic, asphaltic, and naphthenic crudes were prepared with carbon ranges equivalent to C_9-C_{13}, C_{12}-C_{16}, C_{14}-C_{20}, C_{18}-C_{28}, and C_{26}-C_{45+}. Toxicity of aqueous and methanol extracts of each fraction alone or in soil was tested for toxicity to bacterial luminescence and lettuce root elongation. Solid phase tests included earthworm survival and lettuce seed emergence. Results demonstrate that the C_{26}+ fractions were significantly less toxic than the lighter fractions for all trophic levels tested. These results support raising the residual crude oil TEH above the 1000 mg/kg criteria for sites where the C_{26}+ represent the majority of the residual TEH in the soil.

An *In Situ* Respirometric Technique to Measure Pollution-Induced Microbial Community Tolerance in Soils Contaminated with 2,4,6-Trinitrotoluene. P. Gong[1], P. Gasparrini[2], D. Rho[1], J. Hawari[1] and G.I. Sunahara[1]. [1]Biotechnology Research Institute, National Research Council of Canada, Montréal, QC; [2]Department of Chemistry and Biochemistry, Concordia University, Montréal, QC.

Long-term exposure to 2,4,6-trinitrotoluene (TNT) can induce changes in the structure and activities of soil microbial communities. Such changes may be associated with an elevated microbial tolerance. An *in situ* respirometry technique that was based on the analysis of the substrate-induced respiration response to freshly added TNT was applied to examine soil microbial tolerance to TNT at the community level. The specific growth rate derived by fitting an exponential equation to obtained respiration curves was taken as the measurement endpoint. Microbial tolerance was evaluated using an index defined as the ratio of the specific growth rate at a spiking rate of 2,000 mg TNT/kg soil to that of the control with no spiked TNT. Three soils with long-term exposure history (TNT level in soil: 1.5, 32 and 620 mg TNT/kg, respectively) showed significantly higher TNT-tolerance than two uncontaminated control soils. Another soil containing 29,000 mg TNT/kg exhibited the highest tolerance. Findings from the present study further support that the observation of an enhanced community tolerance can be used as a means of identifying those compounds that have exerted selection pressure on the community.

How Do Earthworms Fight TNT Present in the Soil? A.Y. Renoux, M. Sarrazin, J. Hawari and G.I. Sunahara. Biotechnology Research Institute, National Research Council of Canada, Montréal, QC.

The ability of the earthworm *Eisenia andrei* to metabolize 2,4,6-trinitrotoluene (TNT) was demonstrated in experiments with TNT-spiked soils, dermal contact tests, and with an *in vitro* assay. Lethality of TNT in a forest sandy soil was first determined (14 d LC_{50} = 143 mg/kg). Then, TNT at lethal and sublethal concentrations was applied to the same soil and was monitored along with its metabolites in extracts of soil and earthworm tissue for up to 14 d post-application. HPLC-UV analyses indicated that TNT was transformed in *E. andrei* by a reductive pathway to 2-amino-4,6-dinitrotoluene (2-ADNT), 4-amino-2,6-dinitrotoluene (4-ADNT), 2,4-diamino-6-nitrotoluene (2,4-DANT) and 2,6-diamino-4-nitrotoluene (traces) in earthworm tissues. TNT concentrations disappeared from the spiked soils; however, the monoamino-dinitrotoluene (2-ADNT and 4-ADNT) concentrations increased with exposure duration and were dependent upon the initial TNT soil concentration. This was also observed to a lesser extent in the TNT-spiked soils with no earthworms present. The biotransformation of TNT into 2-ADNT, 4-ADNT and 2,4-DANT in *E. andrei* after dermal contact on TNT-spiked filter paper showed that dermal uptake is a significant route of TNT absorption. *In vitro* experiments showed that worm homogenate could metabolize TNT and form 2-ADNT and 4-ADNT at room temperature and at 37°C. This effect was inhibited by heat inactivation prior to incubation or by incubation at 4°C, suggesting that the biotransformation of TNT in *E. andrei* may be enzymatic in nature.

Terrestrial Ecological Risk Assessment of Petroleum Hydrocarbon Contaminated Soils. M.J. Tindal, J.H. Sevigny, G.L. Robins and W. Stein. Komex International Ltd., Calgary, AB.

Ecological risk assessments estimate the likelihood of effects of human actions on ecological receptor organisms and ecosystems. The standard risk assessment paradigm includes the following elements: problem formulation (including identification of chemicals of concern; receptors of concern; and exposure pathways by which the receptors can contact the chemicals of concern), exposure assessment (in which rates of chemical exposure are estimated), toxicity assessment (in which the toxic potency of the chemicals of concern are determined), and risk characterization (where the likelihood of adverse effects is evaluated). Several elements within this risk assessment paradigm are specific to the chemicals of concern. Two challenges specific to petroleum hydrocarbon risk assessments are as follows: petroleum products are highly complex mixtures of organic compounds, with widely differing properties; and the methods and data required to complete a risk assessment for petroleum hydrocarbons are poorly developed relative to other "higher profile" contaminants, such as metals, PCBs and pesticides. Areas of the risk assessment paradigm where these challenges are manifested will be discussed, highlighting solutions and apparent data gaps in the current literature. In particular, the following elements are addressed: selecting hydrocarbon groups; using analytical data to determine the concentration of each group; toxicological limits for hydrocarbon groups; and estimating food chain bioaccumulation and biomagnification.

Assessment of Industrial Discharges

Ecotoxicity Assessment and Remediation of Firefighting Training Wastewater. H. Fanous and I.J. Young. Department of National Defence, Ottawa, ON.

An evaluation of the toxicity and chemical parameters of aqueous film forming foams (AFFF) wastewater associated with the CF firefighting training facility was undertaken as part of an investigation of environmental impact of chemical waste releases from DND operations. Results of the study suggested that firefightng training wastewater was toxic to the aquatic environment-vertebrate, invertebrate and plant species; the acute Microtox® test (IC_{25} endpoint, not IC_{50}) could be used to screen alga toxicity of wastewater; and the one stage, reverse osmosis (RO) membrane treatment system with appropriate pretreatment and cleaning regime was effective at eliminating/ reducing toxicity of wastewater and allowed direct discharge of the permeate (65% of wastewater) to the environment. The RO membrane technology was cost effective over incineration and traditional hazardous waste disposal.

Effect of Aluminum Acclimation on Cardiac Output and Swimming Performance of Rainbow Trout. E.B. Dussault and R.S. McKinley. Department of Biology, University of Waterloo, Waterloo, ON.

Acclimation of salmonids to Al in acidic, soft water may induce a reduction in swimming capacities. Al induced gill damage and other modifications in gill structure may result in decreased oxygen uptake and hence cause an earlier oxygen debt when sustained swimming is required. We attempt to demonstrate these changes using cardiac telemetry. Preliminary experiments have shown that exposure to different concentrations of Al in soft water (20, 40, 80 and 160 µg/L) has an effect on the cardiac output of rainbow trout (*Oncorhynchus mykiss*). Trout (~ 300 g) were acclimated to synthetic soft water (Ca ~50 µM) for 8 wks. They were acclimated to various combinations of pH and Al concentrations (pH 6.5/Al 0 µg/L, pH 5.0/Al 0 µg/L, pH 5.0/Al 20 µg/L, pH 5.0/Al 40 µg/L and pH 5.0/Al 80 µg/L) for four wks. Their swimming performance (Ucrit 10 min) was evaluated while swimming in a modified Blaska swimming chamber. Cardiac surgery was performed and cardiac output was monitored at different water velocities. Blood samples were taken and analyzed for hemoglobin and plasma Al, ions, hematocrit and lactate concentration. Gill and liver were sampled and analyzed for Al. There was a significant effect of water concentrations of Al on swimming performance ($p=0.027$) and gill aluminum accumulation ($p=0.001$). The cardiac output of the control fish (expressed as % basal) increased to 140% at the beginning of the swimming exercise; this was not observed in the fish acclimated to 80 µg/L Al, possibly because of a higher respiratory demand due to Al induced thickening of the gills. The heart rate of fish acclimated to 80 µg/L Al was higher than the control fish at the beginning of the swimming exercise, but did not increase by much as water velocity was increased. The heart rate of control fish was initially lower, and increased to values similar to the fish exposed to 80 µg/L Al at the end of the swimming exercise. These results also suggest fish exposed to Al have a higher respiratory demand in order to obtain the same amount of oxygen from the blood. When sustained swimming is required, fish exposed to 80 µg/L Al do not seem to be able to adjust their cardiac output in the same manner as unexposed fish. We intend to correlate any differences in cardiac output and swimming performance with water chemistry and physiological parameters.

Multidisciplinary Approach for Evaluation of Salt Impacts on Wetland Ecosystems. T.L. Knafla[1], C.T. Oishi[2], M. Bowles[2] and M. Brewster[2]. [1]T.L. Knafla, Calgary, AB; [2]Komex International Ltd., Calgary, AB.

Produced water releases from three oil and gas facilities and process water discharge from a former fertilizer manufacturing facility have resulted in salt-laden water impacts to a diverse group of aquatic environments. Currently, inadequate guidelines are available in Alberta to evaluate the potential for these releases to cause a significant adverse effect. As a result, a novel approach was used to evaluate ecological risks associated with these impacts. The purpose of the approach was to identify the species that are most sensitive to salt water impacts and to determine the nature of the adverse effects on these species. The approach involved combinations of the following techniques: field electrical conductivity mapping; laboratory chemical analyses; desktop literature studies; toxicity testing; and, species diversity and health evaluations. Four different ecosystems were evaluated: freshwater creek; black spruce tree dominated wetland and freshwater creek; narrow-leaf cattail dominated wetland; and, a spring-fed pond in a riparian area. Results for these studies indicate that the most sensitive species differed depending on the ecosystem and nature of the salt impact. The implication of this research is that an approach based on a combination of tools can be used to provide a defensible means for evaluating the potential of salt ion impacts on freshwater ecosystems. This information can be used directly in a risk assessment.

Sea Lamprey as a Tool for Monitoring Toxic Chemicals in the Great Lakes. D.M. Whittle, D.C. MacEachen, R.W. Russell and A.A. Carswell. Department of Fisheries and Oceans, Great Lakes Laboratory for Fisheries and Aquatic Sciences, Burlington, ON.

Samples of adult spawning phase sea lamprey (*Petromyzon marinus)* were collected from 14 streams flowing into each of the 5 Great Lakes. These lamprey were analysed as both skinless muscle tissue samples and whole animals for a range of organochlorine pesticides, total and isomer specific PCBs, toxaphene and total Hg. Contaminant burdens varied significantly from lake to lake. Maximum concentrations of Hg and toxaphene were detected in samples from the Lake Superior basin while PCB levels were highest in samples of lamprey from Lake Michigan and Lake Ontario basin streams. With the exception of Hg, concentrations of all other contaminants measured, were less in the muscle tissue portion of lamprey than in the whole animal samples. Contaminant burdens measured during the same time period, in whole lake trout (*Salvelinus namaycush*), the primary host of the parasitic lamprey, were compared to the various lamprey measurements on a lake by lake basis. Only Hg concentrations were consistently greater in lamprey than in the host lake trout. As a consequence of their feeding pattern and unique ability to accumulate contaminants, lamprey may be designated as an alternate monitoring tool to use as an ecosystem indicator of spatial and temporal contaminant trends. They may also serve to identify the presence of trace levels of previously undetected persistent trace toxic substances in aquatic communities.

Endocrine Disrupting Substances

Decision Making on Hormonally Active Substances: US Testing Program. P.V. Cline[1], C. Pammer[2] and R.C. Lee[2]. [1]Golder Associates Ltd., Gainsville, FL; [2]Golder Associates Ltd., Calgary, AB.

The Endocrine Disruptor Screening and Testing Program (EDSTP), administered by the US Environmental Protection Agency (EPA), focuses on providing methods and procedures to detect and characterize endocrine activity of pesticides, commercial chemicals, and environmental contaminants. The program's proposal, released December 1998, was recently reviewed by EPA's Science Advisory Board and FIFRA's Science Advisory Panel. Current data are not considered adequate for most of the estimated 87,000 chemicals in commerce to allow evaluation of all potential risks. The goal of the Endocrine Disruptor Screening Program is to gather the necessary information to identify endocrine disruptors and take appropriate regulatory action.

Current recommendations for screening and monitoring include a battery of short-term assays for rapid and inexpensive screening of chemicals that still need to be validated and replicated. In addition, the development and validation of other biomarkers that screen for potential embryonic and fetal events; monitoring of wildlife as environmental sentinels; additional research on mechanisms and dose response relationships are needed. The initial step in this process is an analysis of the feasibility of using a "High Throughput Pre-Screening" (HTPS) process that can provide a rapid assay of potential binding with the receptor hormone receptors. This would be applied to a large number of chemicals and provide input for the priority setting process for further testing. However, the identification and evaluation of potential endocrine mediated effects is complex. These compounds may function through a variety of mechanisms, in addition to simple receptor binding. Considering factors like direct and indirect effects, mechanisms to regulate hormone levels, and metabolic processes, the testing program needs to cover a wide array of possible issues, limiting the success of the simple binding assay. The potential use of the HTPS for priority setting depends on many factors including the rate of false positives and false negatives. Because the more complete testing process is slow, regulatory actions or public concerns may be raised before other *in vivo* tests can establish that no hormonal activity occurs. Because of the technical issues, the use of the HTPS in priority setting is unclear.

Assuming a process for priority setting is established, chemicals would enter the Tier 1 screening process on a priority basis to undergo a wider range of assays. The following endocrine disruptor mechanisms for the estrogen, androgen and thyroid systems were included in the Tier 1 Screening battery. The *in vitro* (outside live animals) and *in vivo* (in live animals) assays are listed below. *In Vitro* assays include: an estrogen receptor binding or reporter gene assay; an androgen receptor binding or reporter gene assay; and a steroidogenesis assay with minced testis. *In Vivo* assays include: a rodent 3 d uterotrophic assay; a rodent 20 d pubertal female assay with enhanced thyroid endpoints; a rodent 5 to 7 d Hershberger assay; a frog metamorphosis assay; and a fish reproductive screening assay.

The Tier 1 screening assays cover a range of potential effects. EPA is providing initial emphasis on the legislatively mandated components of this Tier 1 Screening battery. Several screening tests have already entered the validation process, and all the screens are targeted for validation by the end of 2002. EPA has indicated that the ecological tests require substantial development and may take five to seven years to validate. Also, as more effective or efficient tests are developed, EPA will examine their suitability for use and possible replacement of tests currently proposed for use in the screening and testing batteries.

In the recent National Research Council's report, "Hormonally Active Agents in the Environment," the scientific issues were reviewed. It was observed that limitations and uncertainties in data reported in current studies could lead to differing interpretations. In some cases, the studies are not well supported and have not been reproducible. The reason for the inconclusive results is related to the fact that determining if a chemical is "hormonally active" is a mechanism related question. That is, scientists are attempting to determine if a chemical affects an element of the very complicated hormonal system.

The identification of a mechanism or potential toxic endpoint, would typically be linked with exposure and other issues to provide risk management options. The National Research Council report highlights considerations for the framework for decisions regarding ecological impacts. These include clarification of endpoints, pyramid of effects, susceptibility, variability and evolution, temporal and spatial scales, and uses of indicators.

The process of decision-making is challenging given the complexity of the science and long timeframe for developing valid data. The timeline for the current US program includes continued test development, initiation of Tier 1 screening in 2001, with the publishing of a rule in 2002. During this time, many decisions are being made for chemicals identified as "suspects." In many cases, these decisions are independent of the estimated potential exposures, reversibility of effects, or the temporal/spatial scales commonly considered for aquatic systems. Because of the political ramifications of being labelled as hormonally active, decisions may be made to alternate chemicals with less defined but possibly stronger adverse effects. Therefore, the testing process to minimize reporting of false positive results becomes increasingly important.

Detailed Endocrine Assessments in Wild Fish Downstream of Pulp and Paper Mills: Application to the Northern Rivers Ecosystem Initiative Endocrine Program. M.E. McMaster[1], L.M. Hewitt[1], G. Van Der Kraak[2], K. Oakes[2], D. Janz[3], C. Portt[4], and K.R. Munkittrick[5]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Department of Zoology, University of Guelph, Guelph, ON; [3]Oklahoma State University, Life Sciences, Stillwater, OK; [4]C. Portt and Associates, Guelph, ON; [5]University of New Brunswick, Fredericton, NB.

Over that last several years we have been conducting detailed endocrine assessments of wild fish exposed to pulp and paper mill effluents at a number of locations. These studies initially identified reductions in gonadal size, increased age to maturation, reduced expression of secondary sex characteristics and reductions in circulating levels of the major reproductive sex steroids. Over the next several years we expanded our studies to examine in more detail other endocrine endpoints in attempts to identify the mechanisms responsible for this altered reproductive function. This included examination of *in vitro* steroid productive capacity, substrate availability, enzyme efficiency, gonadal apoptosis, stress protein (hsp70) expression, circulating and pituitary gonadotropin levels, steroid binding proteins, hepatic and gonadal oxidative stress, circulating vitellogenin levels, hepatic estrogen receptors, gonadal androgen receptors, prostaglandins and steroid metabolism. In more recent studies we have also begun to examine effluents from various mills to determine whether estrogenic or androgenic compounds are present using a number of *in vitro* endocrine assays. Other studies have focused on characterizing active compounds accumulated in fish from short term and lifetime exposures. The development of these competitive receptor binding bioassays now affords the opportunity to study compounds functioning as hormone ligands. For our initial study, methodology was developed for isolating and characterizing components accumulated in liver tissues from male white sucker (*Catostomus commersoni*) exposed to effluent from one of the Bleached Kraft mills that has previously been associated with these reproductive impairments in wild fish. Examples of these findings will be presented, and details of the Northern Rivers Ecosystem Initiatives Endocrine Program will be described. The NREI endocrine program has been developed to address recommendations to the conclusions of the Northern Rivers Basin Study which identified a number of potential sites for endocrine disruption within the basin. This program is supported through the Northern Rivers Ecosystem Initiative and the new Toxic Substances Research Initiative and is a large collaborative study involving the Federal Government, Alberta Environment, McMaster, Guelph, Florida and Michigan State Universities and private industry including Weyerhaeuser, Millar Western, Alberta Newsprint, Golder and Stantec.

Change in the Chemical Properties of Vitellin-like Proteins in Mollusks: a Consequence of Endocrine Disruption? F. Gagné and C. Blaise. Environment Canada, St-Lawrence Centre, Montréal, QC.

Vitellin-like proteins (Vn) are the major egg-yolk proteins and constitute an important energy reserve for the embryos. Vn synthesis has been demonstrated to be under the control of the estrogen receptor in fish, and more recently in clams. The purpose of this study was to study whether the relative levels of sugars, lipids, phosphates, and labile Zn, normally associated with Vn, could change in clam populations living at contaminated sites. Clams were collected at three sites in the Saguenay fjord region: a marina harbour at Baie-Éternité (BE), a municipal discharge zone at Anse St-Jean (ASJ) and a reference site at Anse St-Étienne (ASE). The condition factor (ratio of wet weight/length^3), sex, metallothioneins (MT), cytochrome P4501A1 activity and DNA damage were determined in the digestive gland. Moreover, the levels of total sugars, lipids, alkali-labile phosphates, proteins, and labile Zn were determined in female gonad homogenates and in purified Vn. The results show that the condition factor was significantly lower at BE site as compared to the reference site with a concomitant increase in DNA damage and MT induction at BE and ASJ sites. In fact, the condition factor was significantly correlated with DNA damage ($r=-0.64$) and MT levels ($r=-0.51$). Female gonad homogenates were found to contain higher levels of labile Zn and lower lipid content at BE stations in respect to the reference site while the ones from ASJ municipal discharge had much higher levels of alkali-labile phosphates (ALP). ALP are well known to be an indirect measurement endpoint for vitellogenin levels in fish and clam plasma (i.e., hemolymph) and was shown in earlier studies to be induced in both the hemolymph and gonadal tissues upon estradiol or nonylphenol (a xenoestrogen) injection. Vn from BE had significantly higher labile Zn but lipid content appeared to be somewhat lower in respect to the reference site. Vn from ASJ were found to be highly phosphorylated in ALP and had higher levels of lipids. These results suggest that female gonad homogenates and Vn lipid, ALP and labile Zn content are altered in clam population subjected to different environmental stresses. The levels of sugars did not change in female gonad homogenates and Vn. The increase of Zn in Vn may represent a pathway for heavy metal transfer to the offsprings.

The Use of the Short-finned Eel (*Anguilla australis*) to Monitor Environmental Health in New Zealand. L. Tremblay, J. Ataria and K. O'Halloran. Centre for Environmental Toxicology (CENTOX), Landcare Research, Lincoln, New Zealand.

Because of its low density of population and green spaces, New Zealand enjoys the reputation of an environmentally clean country. In order to maintain this status CENTOX developed a series of bioindicators and biomarkers to characterise and monitor New Zealand environment for pollution problems. As part of this programme, the short-finned eel is being evaluated as a national bioindicator of aquatic health in New Zealand. The eel was selected because of its high commercial and cultural value. It occupies a high position in the food chain and is widespread throughout the country. To evaluate the presence of estrogenic compounds, a specific eel vitellogenin ELISA was developed. A suite of biochemical endpoints including MFO, immune responses and vitellogenin are being evaluated and validated for their usefulness as biomarkers. Husbandry techniques have been refined and eels can now be successfully kept in the laboratory. Laboratory exposures using estrogenic and MFO inducing compounds have been used to verify whether the eel responds to contaminants that cause specific biological responses. A cage was designed and tested for its efficacy to hold eels. Following it, fish were caged and the biomarkers responses showed significant differences between a clean and a suspected contaminated sites. Therefore this demonstrated the potential for this model as a national bioindicator of environmental health.

Assessing Impact of Municipal and Pulp Mill Effluents and Biosolids on a Suite of Bioassay Organisms. L.H. McCarthy, W. Choi, R. Alvarez and J. McCullam. Departments of Chemical, Biological and Chemical Engineering, Ryerson Polytechnic University, Toronto, ON.

While research was conducted to assess the endocrine-modifying effects of contaminants in sewage treatment plant and pulp mill effluents, the potential impact of chemical compounds from the biosolids in the secondary lagoons was also examined. The link between contaminants in the sediment and possible migration into the overlying water column is obvious. Furthermore, the potential use of biosolids for land application makes assessment of toxicological impact crucial. The current study examined the impact on selected biota of the effluent and biosludge from the Toronto Main Sewage Treatment plant and a bleached kraft pulp mill. The female mosquitofish *Gambusia affinis*, which has shown physiological and behavioral trends towards masculinization when exposed to endocrine-modifiers, was incorporated in the bioassays, while the zooplankter *Daphnia magna* was utilized in 21 d tests. Although these organisms are not strictly benthic, their propensity for intimately associating with the sediment-water interface make them useful indicators of sediment toxicity and potential reproductive modification. To assess the impact of land application of the biosolids, experiments are ongoing utilizing the earthworm *Lumbricus terrestris* and the dicotyledon *Lactuca sativa*. The development of the masculine anal fin in the female mosquitofish exposed to the municipal and industrial effluent was readily observable within a few weeks.

Potential Endocrine Disruption in Freshwater Systems near Agricultural Areas on Prince Edward Island. M.A. Gray[1], K.L. Teather[2], J.P. Sherry[3], M.E. MacMaster[3], R. Mroz[4], W.R. Ernst[4], L.M. Hewitt[3] and K.R. Munkittrick[1]. [1]Biology Department, University of New Brunswick, Fredericton, NB; [2]Biology Department, University of Prince Edward Island, Charlottetown, PE;

[3]Environment Canada, National Water Research Institute, Burlington, ON; [4]Environment Canada, Environmental Protection Branch, Dartmouth, NS.

A pilot study was undertaken in the summer of 1998 to assess whether sediments and freshwater in agricultural areas on Prince Edward Island had the potential to induce endocrine disrupting effects. In the first part of the study, rainbow trout (*Oncorhynchus mykiss*) were caged in eight Prince Edward Island rivers in regions of low, medium, and high agricultural intensity for 21 or 42 d, from mid-July to mid-September to ensure exposure during the most active pesticide spray period . There were three exposure periods: Group I (weeks of July 13-August 3), Group II (weeks of July 27-September 7) and Group III (week of August 24-September 14). Each of the eighteen caging locations were assigned a relative risk ranking based on the assumed potential to receive agricultural run-off. Both male and female fish were analyzed for levels of circulating testosterone (Fig. 1). Although there were some sites with significantly increased and decreased levels of the steroid hormone, there was no clear correlation between testosterone levels and agricultural activity.

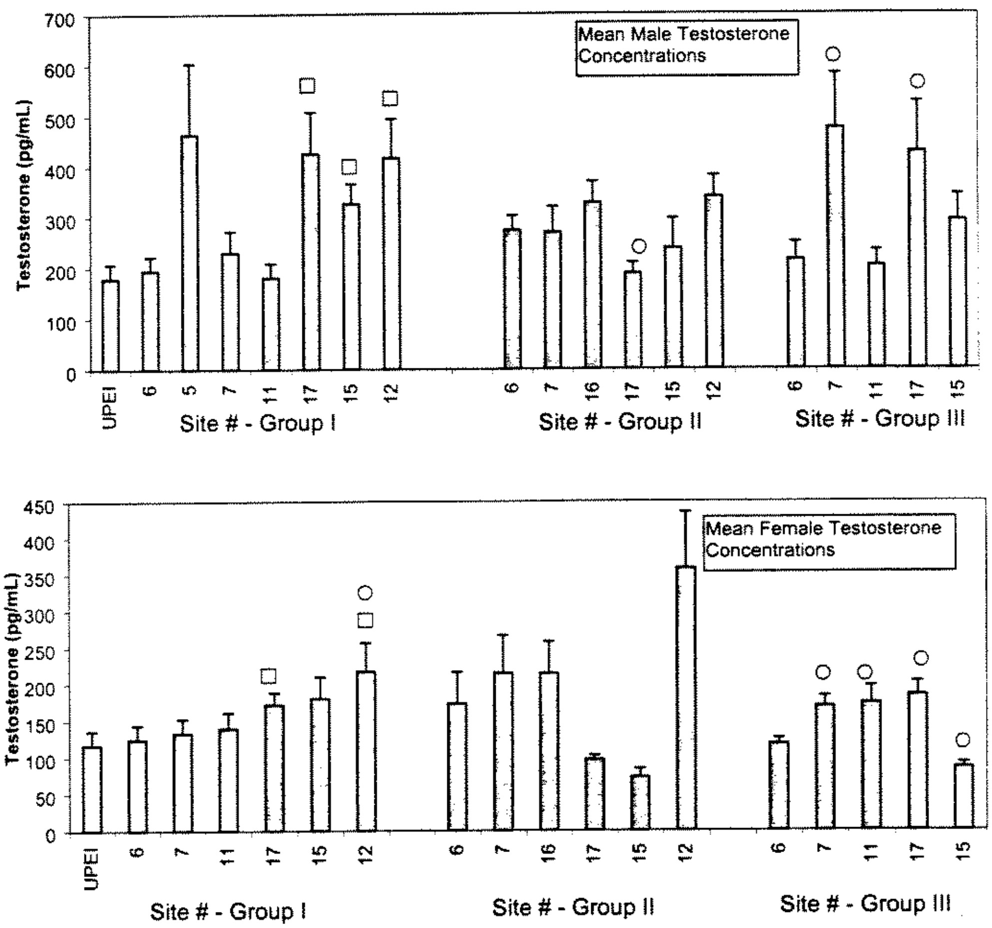


Fig. 1. Mean testosterone concentrations (±SE) in male and female rainbow trout from the three exposure periods (Groups I, II and III). Sites are listed in increasing risk to presumed agricultural run-off exposure. O indicates a signifiacnt difference from the lowest risk site (Site 6) within each group. □ indicates a significant difference (p<0.05) from UPEI fish (Group I only).

In the second part of the study, Japanese medaka (*Oryzias latipes*) embryos were exposed to sediments collected from each of the caging sites. Mortality and failure to inflate a swim bladder was higher in eggs and larvae exposed to sediments from high intensity sites compared with low intensity sites (Fig. 2). Larvae exposed to high risk sediment also took longer to hatch than those exposed to low risk sediment (Fig. 3).

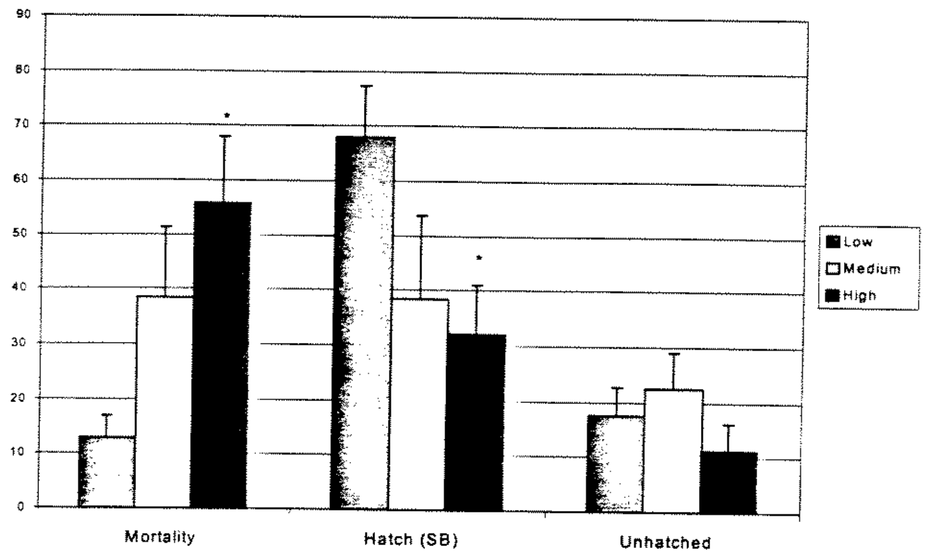


Fig. 2. Mean percent mortality, swim bladder inflation and unhatched larvae (±SE) in medaka embryos exposed to sediments collected from rainbow trout caging sites grouped into low, medium and high risk groups. The * indicates a significant ranking difference ($p<0.05$) from the lowest risk group.

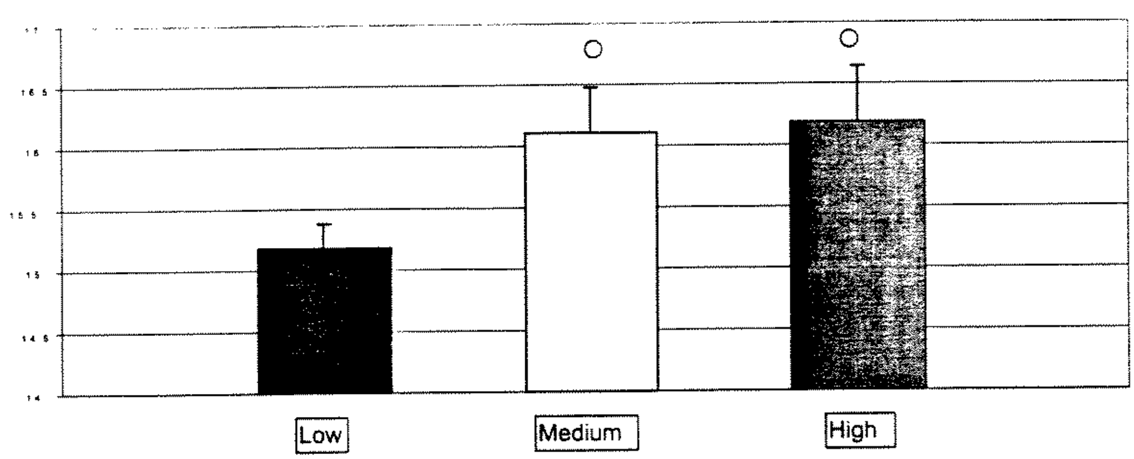


Fig. 3. Mean days to hatch (±SE) for medaka embryos exposed to sediments collected from rainbow trout caging sites grouped into low, medium and high risk groups. Oindicates a significant ranking difference ($p<0.05$) from the lowest risk group.

Concurrent with the caged fish study, a selected number of wild fish were also collected from various rivers across the Island. Some fish were sacrificed and gonads removed to determine if there was evidence of intersex and some fish were anesthetized, blood samples collected and subsequently released back into the river. After histopathological examination, there was no evidence of intersex in any of the gonads. The blood samples were analyzed for circulating levels of testosterone and there were no significant differences between fish collected from high intensity sites compared with those from lower intensity agricultural areas.

While the observed effects in the caged fish component could not be positively attributed to endocrine disruption, the results of the study provided support to undertake additional research activities on PEI in the Summer of 1999. Eight sites were selected on the Island (six in intensive agricultural areas and two in a less intensively farmed area) and at each site, a suite of bioassays was undertaken including the measurement of endocrine responses in caged fish, screening of water and semi-permeable membrane device extracts for endocrine responses and determination of fate and exposure of specific EDCs. Composite water sample were collected on XAD resin for pesticide analysis. The pesticide analyses will provide an indication of exposure levels within each watershed, facilitating comparison of the results of the other assays and aided in the overall assessment of causative agents of the observed effects.

Ten Reasons to be Concerned About Endocrine Disruptors. J. Langer. World Wildlife Fund Canada, Wildlife Toxicology Program, Toronto, ON.

The world's biological diversity cannot be conserved in an environment filled with harmful chemicals. Since the advent of the chemical industry in the 1940s, thousands of chemicals have been produced and released into the air, water and food. Chemicals now contaminate even the remotest parts of the globe and the newly born. Over the past 10 yrs, another hazard associated with chemicals used in industrial processes, consumer products and pesticides has come to light. Research and field data indicate that a wide range of substances have the potential to interfere with the normal functioning of the body's hormone system. Hormones play a particularly important role in the proper development and healthy growth of the developing fetus and newborn. Evidence that synthetic chemicals can impair the offspring as a result of *in utero/in ovo* exposure, and leave the mother unharmed, has been documented in both wildlife and human populations. This has fundamental implications for fertility and reproduction, intelligence, immune defence, and perhaps ultimately, survival. Outlined below are ten reasons for society not only to be concerned about endocrine disruptors, but also to act on them: [1] the endocrine system is universal and essential to all life on earth; [2] the consequences of disrupting the endocrine system are serious and irreversible; [3] a wide range of chemicals, and therefore a wide range of industries, products and activities, are implicated; [4] wildlife are the "canaries in the coal mine;" [5] humans are not immune to the effects of endocrine disrupting chemicals; [6] endocrine disruption can happen at incredibly low levels of exposure, and timing is crucial; [7] neither industry nor government inspire great confidence that they will respond proactively to protect health and the environment; [8] the weight-of-evidence available says "take precautionary action;" [9] endocrine disruption does not damage the genetic blueprint, so eliminating exposure eliminates effect; and [10] it is eminently possible to eliminate endocrine disrupting chemicals.

Perspectives on Endocrine Disrupting Substances in Environment Canada Regulatory Programs. P. Cureton and R. Sutcliffe. Environment Canada, Commercial Chemicals Evaluation Branch, Hull, QC.

Environment Canada's national strategy (Environment Canada 1999) for addressing endocrine disrupting substances in the environment focuses on 4 major elements, namely: national leadership on endocrine disrupting substances national and international harmonization of screening and testing protocols establishing a better knowledge of effects of EDSs in ecosystems assessment/action on priority substances. Specifically regarding priority substances assessments, the *Canadian Environmental Protection Act* authorizes the Ministers of Environment and Health to determine whether substances on the Priority Substances List (PSL) are "toxic" to human health or the environment. The final assessment of CEPA "toxic" considers the entry of the substance into the environment, the exposure concentrations and the levels at which acute or long-term harmful effect may occur. The current assessment regime allows for data on endocrine effects to be considered in much the same way as other more traditional endpoints of acute and chronic toxicity. Indeed many of the toxicological endpoints used in Priority Substances Assessments are related to survival, growth or reproduction, many of which may be modulated by the endocrine system. While EDS data can be evaluated under the current legislation, developments in the science and results from national and international testing and field research will also be factored in to the assessment of existing chemicals. An example of Priority Substances List assessment using endocrine disrupting effects will be discussed.

Polymer Films for Controlled Delivery of Contaminants in Toxicity Tests. R.S. Brown[1], I.S. Kozin[1] and J.C. Klamer[2]. [1]Department of Chemistry and School of Environmental Studies, Queen's University, Kingston, ON; [2]RIKZ (Institute of Coastal and Marine Management), Haren, The Netherlands.

Tests of environmental toxicity often involve compounds which are insoluble and very nonpolar. This poses serious problems when accurate aqueous concentrations of toxicants are to be used. Several processes result in deviations from the "nominal" concentration, including precipitation, adsorption onto vessel walls, and absorption (or partition) into biological tissue. We have developed an alternative method for toxicant delivery wherein a polymer film containing the analyte compound is applied to the sample vessel, and the analyte desorbs from the film into the solution. The amount of material which desorbs is negligible in terms of the total amount in the film, so the solution concentration is constant, and losses from solution are compensated by further desorption from the film. Films were prepared in glass vials and in microtitre plates containing compounds including benzo[*a*]pyrene, aminopyrene and acridine. Equilibrated solution concentrations were linearly proportional to film concentration until approaching the aqueous solubility, where solution concentration leveled off. Concentrations were stable for days, except for cases such as aminopyrene, where instability of the compound restricted the overall stability to several hours. Equilibration times were typically one to two hours for unstirred solutions, but decreased to under five minutes with orbital shaking. Films delivering acridine concentrations from 50 nM to 5000 nM were prepared, and Mutatox® results agreed with those for standard solutions of the same dilution series. Recent experiments with other compounds and toxicity tests will be described.

Profiling Immunotoxicology

Profiling Immunotoxicology - Chairpersons Summary. J.T. Zelikoff[1] and P.T. Thomas[2]. [1]New York University School of Medicine, New York, NY; [2]Covance Laboratories, Inc., Madison, WI.

The purpose of this workshop was to introduce the concept of immunotoxicology to the audience, review validated testing approaches that already exist, describe field studies in which the immune system is studied and provide examples of how environmental chemicals can adversely impact immunity in marine species.

Dr. Peter Thomas provided the initial overview of structure and function of the immune system and described some of the early interlaboratory validation efforts leading up the present testing schemes. In order to be useful for safety and risk assessment, the tests must be relevant to the species in question (be it animal or, ultimately human), robust, sensitive, specific and adaptable to dose-response studies. At the present time, regulatory agencies in the United States and European Union have adopted this general approach to validation and testing. Depending upon the regulatory body, functional tests of immunity are required initially or are triggered from other studies that indicate the immune system as a target organ.

Dr. Jeff Vos followed the introductory presentation with an informative overview of the different types of aquatic field studies that are performed along with a discussion of their strengths and weaknesses. A comprehensive review of comparative responses among aquatic species was also presented using data obtained in flounder (*Platichthys flesus*) exposed to halogenated aromatic hydrocarbons and heavy metals. The take home message was that large interspecies differences in responses exist and well-controlled field or semi-field studies can minimize this variability.

Dr. Vicki Blazer described the use of macrophage aggregates as potential biomarkers of exposure and discussed theories as to their appearance and function in teleost fish. Thought of as primitive lymph nodes in that phagocytized materials is transported to these areas, increases in their appearance, size and color have been correlated with chemical contamination in fish. To support this hypothesis, results were presented from studies with arsenic and dieldrin-exposed fish.

Dr. Judith Zelikoff provided a comprehensive overview of the evidence for immune modulation in fish and justified the use of immune function endpoints as relevant and validated biomarkers of effects. Data from a variety of laboratory exposure studies employing Japanese medaka (*Oryzias latipes*) and bluegill sunfish (*Lepomis macrochirus*) as well as feral fish populations exposed to PCB mixtures were presented. The results suggest that immune assays validated in mammals can be used successfully to demonstrate potential immune modulation in fish populations.

Dr. Tracy Collier provided additional compelling examples of immune disfunction in fish exposed to pollutants. Comparative field studies in juvenile chinook salmon from polluted or relatively clean Puget Sound estuaries demonstrated significant differences in resistance to *Vibrio anguillarum*, a marine bacterial pathogen. Studies conducted in the laboratory using hatchery raised juvenile salmon exposed to polycyclic aromatic hydrocarbon mixtures confirmed the field studies and suggest that pollution can adversely impact salmon populations by modulating host resistance to infectious disease.

Dr. Keith Grasman assessed the impact of persistent organochlorine contaminants on the immune system of Great Lakes fish-eating birds; demonstrating that the impact of man-made industrial chemicals on immunity is not limited to directly exposed aquatic species. Concentrations of contaminants in egg samples and in individual birds was, in many cases, correlated with impaired development of immunologic organs as well as with T-cell function.

Dr. Peter Ross provided other examples of how other species living in marine ecosystems are adversely impacted by persistent environmental chemicals. Following the extensive phocine distemper virus-related mass mortality of harbor seals in northern Europe in 1988, follow up studies using contaminated herring from the Baltic Sea demonstrated that similar contaminants were immunosuppressive to captive-fed seals. The evidence that higher marine mammals demonstrate significant body burdens of halogenated aromatic hydrocarbons suggests that their health is at risk due to potential immune dysfunction.

Dr. Robert Luebke expanded on the introductory presentation by presenting an overview of the laboratory animal data suggesting that pesticide exposure modulates the immune system. Despite the compelling evidence that pesticides are immunomodulatory in laboratory studies, the link between exposure and immunotoxicity in humans is far less clear. By contrast, there are good data to support the contention that it is hypersensitivity, not immune suppression, that is the major issue in human exposure events.

Dr. Marshall Adams concluded the workshop by discussing the importance of immunotoxicological biomarkers in the overall assessment of environmental stress on aquatic ecosystems. Relationships among responses of sentinel fish populations exposed to contaminants at various levels of biological organization (biochemical/physiological, individual, population and community) were presented. Both individual and integrated response analyses suggested that immunological endpoints were sensitive to contaminant stress and demonstrate relevance to higher levels of organization. Therefore, immunological biomarkers of exposure appear to be useful in the ecological risk assessment framework.

Approaches to Immunotoxicity Testing. P.T. Thomas. Covance Laboratories Inc., Madison, WI.

The impact of inadvertent environmental chemical immune dysregulation is receiving increasing attention by the scientific and regulatory community. Furthermore, recent passage of the *Food Quality Protection Act* by the US Congress has focused this issue on children and the unborn fetus. It is for these reasons that considerable attention has been focused on the applicability and predictability of laboratory animal-based assays for immunotoxicity in safety assessment studies. In an early effort to determine the utility of incorporating toxicologic pathology, functional assays of immunity and host challenge into standardized subchronic safety assessment studies, the National Toxicology Program sponsored a multilaboratory validation exercise in which performance of over 15 functional tests and challenge assays was evaluated. Furthermore, to improve risk assessment, the degree to which immune function correlated with host challenge assays was determined. The results of this effort demonstrated that: the degree of correlation between host challenge models and immune function assays was good; and certain combinations of assays were good predictors of immunotoxicity in rodents. The toxicologic pathology and immune function tests represent immunologically relevant endpoints known to be common to mammalian species and to some degree, to those in aquatic environments. Furthermore, the data from these and other validation studies form the basis for current EPA and OECD regulatory guidelines in immunotoxicology. In order for this approach to be applicable for use in the field and to make meaningful risk assessments, issues such as exposure, dose and comparative toxicokinetics must be taken into account during interspecies extrapolation.

Comparative Sensitivity of Different Species to Immunotoxicity Induced by Chemicals of Environmental Concern. J.G. Vos[1,2], G.C.M. Grinwis[2], A.D. Vethaak[3] and P.W. Wester[1]. [1]National Institute of Public Health and the Environment, Bilthoven, The Netherlands; [2]Faculty of Veterinary Medicine, Utrecht University, The Netherlands; [3]National Institute for Coastal and Marine Management, Middelburg, The Netherlands.

The use of toxicity data on environmental chemicals obtained in laboratory animals for risk assessment of wildlife species poses difficulties when species differ qualitatively in target organs of toxicity or interspecies variation in sensitivity to the contaminant is high. Examples of chemicals for which data in wildlife populations are necessary for an adequate understanding of the immunotoxic risks include: PCDDs and PCBs, compounds that are immunotoxic in all species studied, but with extremely large interspecies differences in toxicity; and the organotin TBTO for which thymotoxicity is species-specific. Recently we investigated whether PCDDs, PCBs and TBTO are (immuno)toxic to the flounder (*Platichthys flesus*), a flatfish species common to European coastal areas, estuaries and large rivers. Field and semi-field studies indicated a link between water/sediment contamination, lymphocystis virus infection and liver tumor occurrence. The causal role of immunotoxicants in these diseases was investigated in a study with flounder kept in the laboratory. Exposure to high levels of TCDD or PCB-126 caused liver enlargement, induction of cytochrome P450 and thymus atrophy, results that indicated that flounder is relatively insensitive to these chemicals. Exposure of flounder to TBTO, in concentrations that were in the same order of magnitude as upper TBT field levels, caused mortality associated with gill lesions, and induced thymus atrophy and suppression of the non-specific resistance indicating a possible role of TBTO in the increased prevalence of viral disease. From the present and earlier studies it is concluded that for certain chemicals large interspecies differences in immunotoxicity are found, showing the important role in risk assessment of semi-field or laboratory studies with the species of concern.

Macrophage Aggregates as Biomarkers of Exposure: From Feral Populations to Laboratory Models. V.S. Blazer[1], J.W Fournie[2], C.J. Schmitt[3] and B. Wright[4]. [1]National Fish Health Research Laboratory, USGS/BRD, Kearneysville, WV; [2]US EPA, Gulf Division, Gulf Breeze, FL; [3]Columbia Environmental Research Center, USGS/BRD, Columbia, MO; [4]AScI, c/o USGS-CERC.

Macrophage aggregates (MAs) are structures within the spleen, kidney and sometimes liver of teleost fishes. They are believed to function much like primitive lymph nodes in that phagocytized material is transported to these areas by macrophages, for destruction, recycling or storage. Initiation of the specific immune response may take place as the macrophages migrate to these structures. For many years researchers have noted increased number, size and/or pigment content of MAs in fish collected from contaminated versus reference sites. Hence, they have been used as a biomarker in the USEPA's EMAP (Environmental Monitoring and Assessment Program) and well as USGS's BEST (Biomonitoring of Environmental Status and Trends) program. Results from the EMAP-Estuaries program in the Gulf of Mexico have suggested that increased MAs are one of the best bioindicators of sediment contamination in bottom-dwelling fishes. Laboratory exposures to individual contaminants have begun for validation of this biomarker as well as to increase our understanding of the mechanisms of response and relationship to immune status. Results from exposures to arsenic and dieldrin will be presented. In addition, confounding parameters such as age and infectious disease will be discussed.

Immunotoxicity Biomarkers in Fish: Development, Validation, and Application to Field Settings. J.T. Zelikoff[1], E. Carlson[1], Y. Li[1], A. Raymond[1], J.R. Beaman[2] and M. Anderson[3]. [1]New York University School of Medicine, New York, NY; [2]Geo-Centers Inc. Frederick, MD; [3]California EPA, Sacramento, CA.

Immunocompetence is usually monitored using a tiered approach that is based upon several parameters including immunopathology, immune function, and host resistance. Through the efforts of a number of different laboratories, well-characterized immune assays validated in rodents for their sensitivity and reproducibility in assessing xenobiotic-induced immunotoxicity are currently available. Many of these same assays have been developed and used as biomarkers to predict chemical-induced immunotoxicity in animal species other than mammals. In this laboratory, immune assays that measure immunopathology, antibody-forming cell response to T-dependent antigens, T- and B-lymphocyte proliferation, macrophage function and antioxidant activity, as well as host resistance against infectious bacteria have been employed successfully to assess metal, pesticide, polycyclic aromatic hydrocarbon (PAH), and chemical mixture-induced immunotoxicity in laboratory-reared Japanese medaka (*Oryzias latipes*) and bluegill sunfish (*Lepomis macrochirus*). Many of these same endpoints have also proved successful for predicting the immunotoxicity of contaminated aquatic environments in feral fish populations. For example, smallmouth bass collected from a polychlorinated biphenyl (PCB)-contaminated site demonstrated significant reductions in kidney phagocyte function and antioxidant levels compared to that measured in reference site fish. Results of the aforementioned studies demonstrate that immune assays validated in mammals can be used successfully in a small aquaria fish to demonstrate the immunomodulating effects of environmental chemicals, as well as for biomarkers to predict the toxicological hazards associated with "real world" polluted aquatic environments. (Supported by U.S. Army Center for Environmental Health Research, Contract No. DAMD-17-93-3059.)

Immunotoxicity of Environmental Pollutants: Adverse Effects on Resistance to Infectious Disease. M.R. Arkoosh[1], E. Clemons[1], A.N. Kagley[1], E. Casillas[2], J.E. Stein[2] and T.K. Collier[2]. [1]Environmental Conservation Division/ NWFSC/NMFS/NOAA, Newport, OR; and [2]Seattle, WA.

Wild Pacific salmon populations are in serious decline, and as a result, a number of salmon stocks are listed as threatened or endangered, under the *Endangered Species Act*. Our research supports the possibility that environmental pollutants, can alter salmon survival, and as a result may contribute to these declines. We have shown that juvenile chinook salmon (*Oncorhynchus tshawytscha*) are exposed to polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) as they migrate through a contaminated urban estuary in Puget Sound WA (e.g., the Duwamish Waterway estuary). Immune function was also analyzed in these fish by examining the ability of their anterior kidney and splenic leukocytes to produce a primary and secondary *in vitro* plaque-forming cell (PFC) response to the hapten, trinitrophenyl (TNP) and by determining their susceptibility to a marine pathogen, *Vibrio anguillarum*. We found that fish outmigrating from the urban estuary produced a significantly lower PFC response to TNP and were more susceptible to the pathogen, compared to juvenile salmon collected from a rural estuary during their outmigration. In the laboratory, we exposed juvenile chinook salmon collected from a hatchery to either a PCB mixture or a PAH compound to determine if pollutants in and of themselves have the potential to alter immune function in salmon. Indeed, we found that salmon exposed in the laboratory to either a PCB mixture or a PAH also produced lower PFC responses and were more susceptible to disease, compared to animals treated with the solvent vehicle. In summary, pollutants such as PAHs and PCBs are demonstrated to influence salmon health, and thus have the potential to adversely impact salmon populations.

Organochlorine-Associated Immunotoxicity in Fish-Eating Birds of the Great Lakes. K.A. Grasman. Wright State University, Dayton, OH.

Persistent organochlorines, especially polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, are powerful immunosuppressants in birds and mammals. Our objective was to examine associations between organochlorines and immunosuppression in herring gull (*Larus argentatus*) and Caspian tern (*Sterna caspia*) chicks of the Great Lakes. This study employed biomarkers of immunological structure and function and assessed organochlorine concentrations in pooled egg samples and individual birds. The development of immunological organs was assessed in pipping herring gull embryos and 4 wk old gull chicks. *In vivo* immune function tests were conducted on 3 wk old gulls and terns. In herring gull embryos, there was an association between PCBs and reduced thymocyte (developing T cells) viability and numbers. The mass of the thymus and bursa of Fabricius, and the number and viability of developing lymphocytes in the bursa were not associated with PCB contamination. In gull chicks, thymic atrophy was not associated with organochlorines. However, the phytohemagglutinin (PHA) skin test, an integrative *in vivo* assay for T cell function, was suppressed significantly in gulls from Saginaw Bay, western Lake Erie (2 colonies), and eastern Lake Ontario. The PHA response in terns from Saginaw Bay was suppressed significantly compared to a reference colony in northern Lake Huron, and this response was correlated negatively with plasma PCB concentrations measured in individual birds. These biomonitoring data support our previous studies and indicate organochlorine-associated suppression of T cell-mediated immunity in two species of colonial waterbirds at several contaminated sites in the Great Lakes.

Mass Mortalities in Wildlife: Immunotoxicity or Natural Events? P.S. Ross. Department of Fisheries and Oceans, Institute of Ocean Sciences, Sidney, BC.

Outbreaks of infectious disease in wildlife can be considered complex ecological events, with numerous contributing factors. However, high-profile mass mortalities in recent years, particularly among seals and dolphins, have drawn attention to the possible role of environmental contaminants. Fish-eating marine mammals that inhabit the coastal waters of the industrialized world are exposed to high levels of lipophilic contaminants, including PCBs, dioxins and furans. Many congeners within these classes of compounds can assume planar structures and have "dioxin-like" properties. These "dioxin-like" chemicals have been associated with Aryl hydrocarbon receptor (*Ah*R)-mediated immunotoxicity in mammals. Following the dramatic phocine distemper virus (PDV)-related mass mortality of 20,000 harbour seals (*Phoca vitulina*) in northern Europe in 1988, a captive feeding study found that herring from the contaminated Baltic Sea was immunotoxic to a captive group of seals. The authors concluded that contaminants, and PCBs in particular, contributed to the severity of the event. Taken together, evidence from parallel rodent studies, other immunotoxicological studies using laboratory animals, the captive feeding study of harbour seals, and observations from field studies suggest that free-ranging marine mammals in many parts of the world are at risk to the immunotoxic effects of PCBs. While conclusive studies are ethically unacceptable and would be difficult to design, a "weight of evidence" approach represents a useful alternative. Given the persistence of the PCBs in environmental compartments during the last decade, some wildlife occupying high trophic levels will continue to be at risk for contaminant-related immunotoxicity and diminished resistance to infectious disease for some time.

Pesticide-induced Immunotoxicity: Are Humans at Risk? R. Luebke. U.S. Environmental Protection Agency, Research Triangle Park, NC.

One of the first immunotoxicology studies determined that exposure of ducks to DDT reduced their resistance to a virus infection. The immunotoxic potential of insecticides and herbicides has subsequently been studied extensively in laboratory animals, driven by the global distribution and use of these chemicals. (Ten of the twelve persistent organic pollutants, identified by the United Nations Environmental Program as posing the greatest threat to humans and wildlife, are pesticides; all have been reported to alter immune function under certain conditions.) Human health effect studies suggest that the increased rates of some lymphatic malignancies in individuals with occupational pesticide exposure may be due to direct or indirect effects on cells of the immune system, based on the known immunotoxic effects of cytoreductive therapeutic drugs and certain other chemicals. Nevertheless, our knowledge of the human health risks associated with pesticide use and exposure is far from complete. This presentation will provide an overview of pesticide immunotoxicity studies in animals; the implications that these data have for human health effects will be discussed. Finally, factors that may influence the extrapolation of rodent data to potential human health effects, and the evidence for human immunomodulation by pesticides, will be reviewed. (Disclaimer: This abstract does not represent EPA policy.)

Importance of Immunotoxicological Indicators in Assessing the Effects of Environmental Stress in Aquatic Ecosystems: Potential use within the Ecological Risk Assessement Framework. S.M. Adams and M.S. Greeley. Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN.

Biological effects of environmental stressors usually involve a series of responses ranging from the biomolecular/biochemical to the population and community levels. Bioindicators of stress at lower levels of biological organization (i.e., MFO detoxification and antioxidant enzymes) can provide direct evidence of contaminant exposure while intermediate-level responses such as histopathological, bioenergetic, and reproductive changes can be predictive of effects at higher levels of biological organization. Since immunological responses to stressors are primarily physiological in nature, the question is, can immunotoxicological stress responses be informative indicators of ecologically- relevant changes in aquatic systems and what is their potential contribution within the Ecological Risk Assessment (ERA) framework. To address this important question, field studies were conducted in which spatial patterns in fish responses to contaminant loading were investigated in a stream receiving point-source discharges of various contaminants. Relationships among responses in a sentinel fish population at four major levels of biological organization (biochemical/physiological, individual, population, and community levels) were evaluated relative to patterns in contaminant loading along the spatial gradient of the stream. Both individual and integrated response analysis demonstrated that bioindicators at several levels of biological organization displayed similar downstream patterns in their response to contaminant loading within the stream. Some of the bioindicators at lower levels of organization such as immunotoxicological responses appear to be useful in the ERA process because of their sensitivity to stressors and apparent relationships to higher levels. (Managed by Lockheed Martin Energy Research Corp., under contract DE-AC05-96OR22464 with the U.S. Department of Energy.)

Biomarkers

Sediment Bioassays to Measure the Bioavailability of MFO-inducing Compounds to Fish. T. Chan, S. Zambon and P.V. Hodson. School of Environmental Studies, Queen's University, Kingston, ON.

The risk to fish of polynuclear aromatic hydrocarbons (PAH) in sediments will depend in large part on bioavailability. While there are protocols available for testing the acute toxicity of sediment-borne compounds to aquatic invertebrates and fish, there are none for assessing bioavailability to fish. We have found that sediment-borne crude oil, coal-tar, or pure PAH caused an increase of MFO activity in trout fingerlings exposed in a static 4 d bioassay. Assuming that PAH are taken up by fish due to partitioning from organic and inorganic constituents of sediment, we tested the effect of different test variables on bioaccumulation, using MFO activity as an index of exposure. Factors tested included area vs volume of sediment, sediment characteristics (organic silt vs clay vs sand), mixing and aging of spiked sediments, freezing vs cold-storage of natural and spiked sediments, and establishment of gradients through sediment dilution vs sediment volume. Preliminary results demonstrate that induction varies with the amount of contaminated sediment in a tank in a repeatable way. We will present data comparing sediment factors and characterizing protocols according to precision and relative sensitivity.

Are TEFs a Suitable Model for Estimating MFO Induction in Trout by Mixtures of PAH? N. Basu, S. Billiard and P.V. Hodson. School of Environmental Studies, Queen's University, Kingston, ON.

We tested whether toxic equivalency factors (TEFs) could be used to assess mixed function oxygenase (MFO) induction in rainbow trout by mixtures of polycyclic aromatic hydrocarbons (PAHs). The PAHs, listed in decreasing potency for induction were: benzo[*k*]fluoranthene, benzo[*b*]fluoranthene, 2,3-benzo[*b*]fluorene, and 7-isopropyl-1-methyl phenanthrene. TEFs were derived for each PAH by using B[*k*]F as the reference compound. Juvenile trout (2-3 g) were exposed to single PAH and to binary, tertiary and quaternary mixtures of PAHs at total equivalent concentrations (0.32, 1.0, or 3.2 µg/L B[*k*]F. Exposure-dependent increases in MFO activity were observed in fish. Assuming additivity, MFO activity at a given TEQ should be equal among mixtures at the same total concentration of TEQs. PAH tested singly gave the same MFO

induction as B[*k*]F at the same concentration of TEQs. At a specific TEQ, there were also no differences among MFO activities of fish exposed to mixtures with the same number of constituents. However, mixtures of two constituents caused greater than additive responses (~ 2.4-fold) compared to the single PAHs, and tertiary mixtures caused ~ 3-fold greater MFO activity than single PAHs. In contrast, the quaternary mixture increased MFO induction by only 1.6-fold. In summary, TEFs did not predict MFO induction in PAH mixtures of two to four compounds based on additivity of single PAHs. Thus, risk assessments for mixtures should be based on bioassays rather than on TEFs. Further studies are needed to completely characterize joint effects.

Fish Health Effects from Oil Sands Wastewater Discharges and Naturally-Occurring Oil Sands Compounds in the Athabasca River System. J. Parrott[1], W. Gibbons[2], T. Van Meer[3], M. Baker[1], M. Colavecchia[1] and J.P. Sherry[1]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Golder Associates, Calgary, AB; [3]Syncrude Canada Ltd., Environment Department, Fort McMurray, AB.

To assess the health of fish in the oil sands region, NWRI and the Regional Aquatics Monitoring Program (RAMP) sampled longnose sucker (*Catostomus catostomus*) from the Athabasca River upstream and downstream of the oil sands facilities. Reference fish were captured 240 km upstream (Athabasca River at Iron Point) outside the oil sands formation. The downstream site was below the Suncor wastewater discharge, downstream (40 km) of Fort McMurray. Longnose sucker from the downstream site had MFO activities 10-fold above the upstream site. Livers of fish from the oil sands site were significantly smaller and condition factors (CF) significantly lower than liver of fish from upstream. Male longnose sucker had significantly higher testosterone concentrations that upstream fish, while female longnose sucker had significantly lower testosterone concentrations than upstream fish. There were no significant differences in gonadosomatic indices of oil sands and upstream fish. Fecundity was lower in oil sands fish, and there was a decrease in the number of eggs per gram of ovary compared to upstream fish. Circulating vitellogenin (Vg) in female fish was decreased in the oil sands area. From this preliminary assessment, there appear to be differences in the fish health parameters (liver size, CF, fecundity) of oil sands exposed fish. As well, metabolic bioindicators (MFO) and reproductive bioindicators (testosterone and Vg concentrations) showed difference between oil sands and reference sites. The downstream site receives anthropogenic oil sands refinery input, anthropogenic input from Fort McMurray, as well as natural oil sands-related compounds from exposure to the oil sands formation. Our studies over the next three years will focus on teasing out the contributions of anthropogenic versus natural oil sands compounds.

Summary of Studies Assessing the Hazards of Oil Sands Related Reclamation Waters to Yellow Perch. M.R. van den Heuvel[1], M. Power[2], M.D. MacKinnon[3] and D.G. Dixon[4]. [1]New Zealand Forest Research Institute, Rotorua, New Zealand; [2]Department of Agricultural Economics, University of Manitoba, Winnipeg, MB; [3]Syncrude Canada Ltd., Edmonton, AB; [4]Department of Biology, University of Waterloo, Waterloo, ON.

In order to test the viability of oil sands aquatic reclamation techniques, yellow perch (*Perca flavescens*) were stocked into three experimental ponds. Pond substrates consisted of either oil sands fine tailings, or clay and lean oil-sands deposited by the mining operations. Perch were stocked immediately post-spawning and subsamples were sacrificed at 5 and 11 months to measure indicators of energy storage and utilization. These indicators included: survival, age, spawning periodicity, condition factor, gonad size, fecundity, and liver size. Stocked perch showed an initial decline in numbers in the first few months after stocking. Chemical and biochemical parameters in perch showed a gradient of exposure to oil sands related compounds. Despite this, physiological indicators generally showed patterns consistent with improved energy storage and utilization in the experimental pond perch as compared to perch in the lake from which the stocked fish originated. This was evidenced by increased gonad size, condition factor, liver size and the disappearance of spawning periodicity. The patterns observed in experimental ponds suggest improved resource availability and/or reduced intra- and interspecific competition. Perch physiological indicators fell within the range of those measured at several remote natural lakes in the area. Histological gill lesions and increased rates of disease were observed in the oil sands impacted ponds. Reduced growth and deformities in naturally-spawned YOY perch were noted at some of the sites indicating that effects on early-life-stages of fishes may be of concern for the systems studies.

Identification of Solid-phase Geochemical Fractions Contributing to the Metal Body Burdens of Benthic Invertebrates. J.C. Evans and D.G. Dixon. Department of Biology, University of Waterloo, Waterloo, ON.

The development of reliable models for sediment metal toxicity requires the identification of all sediment compartments that contribute to the total pool of bioavailable metal. Traditional test methods used to examine metal uptake from sediment are unable to separate the contributions to metal body burden from the overlying and pore waters from that of the solid phase. As neither the solid phase nor water phase of sediment can be examined independently, a novel experimental design was used in which metal uptake from water compartments was the same in both control and treated sediments, a recirculating tank in which a single pool of water is aerated and forced through multiple sediment containers. This ensured that metal concentrations in overlying water of each sample container was the same and greatly reduced differences in porewater metal content. This apparatus was used to confirm that Ni and Cu present in the solid phase of treated mine tailings (Falconbridge Ltd., Onaping-Levack) was available to *Hyalella azteca* and *Tubifex tubifex* exposed to a mixture of floc and control sediment in four replicate tanks. Metals (Cu, Ni, Cd) occluded in synthetic geochemical fractions were also found to be available to benthos. Solid-phase metal spikes were considered bioavailable only if gut-purged body burdens of exposed animals were greater than those in the controls, and metal concentration in filtered porewater was the same in spiked and control sediments.

***In situ* Evaluation of the Effects of Stormwater Discharge on Early Life Stages of Cutthroat Trout.** H.C. Bailey[1], F. Landry[1], A. La Grange[1], A.R. Tang[1] and L. Ouellet[2]. [1]EVS Environment Consultants, North Vancouver, BC; [2]BC Hydro, Burnaby, BC.

A series of toxicity tests and toxicity identification evaluations conducted on stormwater discharged from an electrical substation and stores facility indicated that Zn was responsible for the intermittent toxicity that was observed. To evaluate potential effects of the stormwater on the receiving environment, eyed cutthroat trout embryos were placed in hatch boxes in the stormwater discharge ditch and in a creek that the ditch subsequently flows into. Creek sites were located both upstream and downstream of the ditch. A subset of the embryos were also reared in the laboratory to provide a baseline for comparing hatching success, survival, growth and development. The exposure duration was 40 d. No adverse effects were found when the embryos reared in the ditch were compared to embryos reared in the laboratory. However, the embryos at the upstream and downstream receiving environment sites exhibited adverse effects in at least one of the parameters measured compared with the laboratory and ditch treatments. This study illustrates the importance of evaluating water quality in the receiving environment before assigning priorities to reduce inputs of contaminants.

Development of a Human Bioassay to Detect Metals, Organochlorine Pesticides and PCB's in Lake Sediment Extracts. P.F. Dehn, J. Shea and S. Allen. Department of Biology, Canisius College, Buffalo, NY.

HepG2 (human hepatocellular carcinoma cell line) cells were exposed to a 2.5% fraction of sediment extracts for 48 h. Sediment extracts containing pesticides and PCB's were prepared by EPA method 3540c using a methylene chloride-hexane solvent system, fractionated on a florisil column according to EPA method 36.20, concentrated via evaporation to dryness, and then re-dissolved in DMSO. Metals were extracted using EPA method 200.7, and neutralized. Cells exposed to organic fractions were monitored for cytochrome P450 activities (EROD, PROD) and gluthathione (GSH) levels, while those exposed to metal extracts were monitored for induction of metallothionein (MT). EROD levels were measured fluorimetrically while PROD and GSH levels were measured enzymatically. MT levels were determined using the cadmium-hemoglobin binding assay. T-tests (alpha 0.05) were performed between no exposure and solvent control groups and sediment extract exposure and solvent controls. All data was expressed as a percent of the control. EROD (1985%, 1685%, 1745%) and PROD (169%, 165%, 179%) activities and GSH (154%, 152%, 144%) levels were higher in fractions 1, 2 and 3, respectively which contained pesticides and PCB's than in the solvent controls. MT levels were significantly higher (145%) in cells exposed to metal sediment extracts, which contained 20.7, 0.04, and 0.34 mg/kg Pb, Cd and Hg, respectively. Results suggest that this human bioassay can detect real-world mixtures of contaminants in sediments. (Undergraduate researchers JS and SA were supported by an AAAS/Merck Interdisciplinary Research Grant to Canisius College.)

Quantification of PAH Accumulation in Rainbow Trout Sac Fry Tissue using Fluorescence Spectroscopy. S.P. Tabash, S.M. Billiard, S.A. Hawkins, I.S. Kozin, R.S. Brown and P.V. Hodson. School of Environmental Studies, Queen's University, Kingston, ON.

The accumulation of waterborne polycyclic aromatic hydrocarbons (PAHs) by rainbow trout sac fry varies significantly between PAHs. The bioconcentration factor (BCF) of these compounds will be driven by their K_{ow} and rate of metabolism by MFO enzymes. To fully characterize PAH bioaccumulation, several samples at different time periods of exposure must be analyzed. Such analyses using conventional methods (i.e., GC/MS) is both time-consuming and expensive. Using synchronous scanning fluorescence spectroscopy (SFS), however, tissue body burdens of a given PAH in sac fry can be readily determined for large sample sizes. From these results, the uptake of PAHs by sac fry from waterborne PAH exposure can be studied. Body burdens of PAH, including retene, phenanthrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and benzo[*b*]fluorene were determined using SFS. A standard protocol was developed for analyzing PAHs from sac fry tissue involving whole larval homogenization, solvent extraction of the PAH from tissue, and clean up through a fluorosil chromatographic column followed by PAH detection using SFS. Average recovery for retene using the new protocol was 82±9%. The limit of detection for retene and phenanthrene was calculated as 2.3×10^{-9} and 1.35×10^{-9} mol/g dry weight, respectively. The bioconcentration factors for trout larvae exposed to nominal waterborne retene and phenanthrene for ca. 32 d (24 h static renewal) were calculated at <1.68 and 1030. The SFS analysis protocol has a similar sample preparation time to standard methods such as GC/MS, but SFS measurement on the fluorimeter requires significantly less time (<5 min). For samples containing one or only a few PAH compounds this allows for a much larger number of analyses at a much lower cost. This can improve characterization of processes such as BCF and pharmacokinetics in fish in model PAH studies.

Does the Potency of Polycyclic Aromatic Hydrocarbons (PAH) for Inducing CYP1A in Juvenile Trout (*Oncorhynchus mykiss*) **Predict Dioxin-like Toxicity in Early Life Stages?** S.M. Billiard[1], P.V. Hodson[1] and N.C. Bols[2]. [1]Department of Biology, Queen's University, Kingston, ON; [2]Department of Biology, University of Waterloo, Waterloo, ON.

We have shown that chronic exposure of rainbow trout early life stages to an alkyl-substituted phenanthrene (retene) causes dioxin-like toxicity or bluesac disease. The rank order of potency for chlorinated PAH in causing blue sac disease is the same as the relative potency of these congeners for inducing CYP1A. Results suggest that rapidly metabolized PAH share the same mechanism of toxicity as the more persistent and toxic CPAH, i.e., oxidative stress due to prolonged CYP1A induction. If correct, this model predicts that the chronic toxicity of PAH to larvae should increase with their potency for MFO induction and could provide a tool for rapid risk assessment of PAH/mixtures. Using the median effective concentration (EC_{50}) from exposure-response curves, fish-specific toxic equivalency factors (TEF) were derived for several PAH *in vivo*. With the exception of phenanthrene, all PAH tested induced CYP1A activity in juvenile trout and were ranked as follows: benzo[*k*]fluoranthene > benzo[*b*]fluoranthene > benzo[*b*]fluorene > retene. However, short-term CYP1A induction in juvenile trout did not predict chronic toxicity. Phenanthrene, a non-inducer, showed bluesac symptoms at high molar concentrations. Preliminary tissue burden analyses suggest that phenanthrene toxicity could be due to non-polar narcosis. We are currently testing the mechanism of oxidative stress due to prolonged CYP1A induction in retene-exposed eggs/larvae by measuring biochemical indices of oxidative stress and co-exposures to both antioxidants and CYP1A inhibitors.

Integrating Exposure and Effects Endpoints in Laboratory and Field Bioassays: Lessons Learned from Caged Bivalves. M.H. Salazar and S.M. Salazar. Applied Biomonitoring, Kirkland, WA.

Almost 10 yrs ago Lynn McCarty suggested revising standard aquatic bioassay protocols. He identified the need to develop a single bioassay methodology that included direct measurements of exposure and effects. We developed an *exposure-dose-response* triad based on a series of caged bivalve studies measuring bioaccumulation and growth as indicators of exposure and effects. Interestingly, there are still no standard laboratory protocols that include synoptic measurements of exposure and effects in the same test. We believe that the continued use of separate toxicity and bioaccumulation tests results in a significant loss of information and a lack of understanding of the processes affecting bioaccumulation and growth. This also limits the utility of these tests as a predictive tool for real-world applications. In sediment testing for example, standard protocols are available for the *Macoma* and *Nephtys* bioaccumulation tests, but the environmental significance of these data is often unclear. Considering the effort and cost associated with setting up these bioaccumulation tests and performing the chemical analysis, the addition of effects endpoints, such as tissue weights, is relatively minor and yet adds potentially significant information. Similarly, standard protocols exist for *Neanthes* toxicity tests using the growth endpoint but actual exposure at receptors of concern is unknown. This paper will identify: problems in interpreting the environmental significance of results from standard laboratory tests for toxicity and bioaccumulation; advantages in making synoptic measurements; and revisions to standard protocols that toxicity based on water and sediment chemistry, tissue chemistry, and associated biological effects.

Environmental Effects Monitoring: Pulp and Paper

Environmental Effect Monitoring of Pulp Mill Effluent using Salt Water Mussels in an On-Site Bioassay. K. Kinnee[1], C.A. McDevitt[1], K. Hall[2], M. Davies[3] and C. Easton[4]. [1]BC Research, Vancouver, BC; [2]Department of Civil Engineering, University of British Columbia, Vancouver, BC; [3]Hatfield Consultants Ltd., West Vancouver, BC; [4]Fletcher Challenge Canada, Elk Falls Pulp and Paper, Campbell River, BC.

The objective of this study was to determine the effects of pulp mill effluent on the survival and growth of the mussel, *Mytilus edulis*. This study was conducted on site at the Fletcher Challenge Canada Ltd., Elk Falls Pulp and Paper Mill in Campbell River, BC, as part of this mill's EEM. Neither an *in-situ* test nor a wild mussel survey were feasible at the study site because of safety issues associated with the rapid current in the receiving environment surrounding the mill. The mussels (400 per concentration) were exposed to 6 concentrations (0.0, 0.22, 0.46, 1.0, 2.2 and 4.6%) of pulp mill effluent diluted with seawater for 90 d between June and September 1999. Mussels were obtained from a local mussel farm and were sorted into 1 mm size classes and distributed individual cages made from oyster netting. Each individual mussel was monitored over the test period. At the beginning and end of the experiment, whole wet weight and length were measured. At the beginning of the experiment, 400 mussels were sacrificed and tissue and shell weights were measured, to determine baseline conditions. During the experiment, there was daily monitoring of the test conditions (dissolved oxygen, temperature, salinity, and pH) and the concentrations of effluent. At the end of the experiment, tissue and shell weights were measured and condition index was calculated. The experimental set up will be shown, and preliminary data presented.

Effects of Pulp and Paper Mill Effluent on the Reproductive Physiology of Rainbow Trout. M.R. van den Heuvel, R.J. Ellis, N.A. Marvin and T.R. Stuthridge. New Zealand Forest Research Institute, Rotorua, New Zealand.

Published observations of the effects of pulp and paper effluents on the reproductive physiology of fishes include reduced circulating sex steroid hormones, reduced gonad size and increased ovarian apoptosis. Field surveys often have poor exposure data and little control of environmental variables. Laboratory studies tend to be short term, often with unrealistically high concentrations of effluent. There is a need for long-term studies on the reproductive fitness of fishes under controlled exposure and environmental conditions to address some of the controversy surrounding pulp and paper effluent effects. This study undertook effluent exposures of 2+ aged rainbow trout that were approximately half-way through gonadal growth. Trout were exposed to a mixed TMP/BKME effluent in 12,000 L flow-through exposure tanks at a recently constructed environmental research facility located at the Tasman pulp mill in Kawerau, New Zealand. Trout were exposed to either upstream river water or 10% effluent in upstream river water and were maintained at a ration of 0.7% of body weight during the experiment. Results of the 2 mon study indicated that trout survival was not significantly different between effluent-exposed tanks and reference tanks. There was extensive growth during the exposure but no differences were found due to effluent exposure. Gonadal development was not significantly different between treatments. Other physiological indicators of energy storage and utilization also showed no significant differences. Biochemical indicators of adult trout reproductive fitness are discussed.

Application of a Gradient Design to Assess Benthic Community Recovery near a Marine Pulpmill Outfall. G.P. Thomas and N. Munteanu. G3 Consulting Ltd., Richmond, BC.

EEM Cycle 2 assessments were conducted at a coastal pulpmill discharging final effluent to a marine system possessing a complex hydrographic profile and numerous point and non-point source confounding influences. The ECF Kraft mill had undergone process and effluent treatment improvements in recent years. The previous Cycle 1 design (control/impact) was ineffective in adequately assessing any associated pulpmill effects on the receiving environment. Cycle 2 research applied an effective and insightful Gradient Design focussed on substrates and associated benthic invertebrates communities. This paper discusses how indigenous benthic communities successfully delineated, through community structure endpoints, the extent, magnitude and direction of exposure to mill discharge while separating these findings from system effects and confounding influences. The extent of recovery exhibited by the local communities through the assessment of indicator species, community structure trends, community associations, faunal similarity analysis and sediment cluster correlations will be examined. Other endpoints such as biomass, species richness and total abundance are also discussed. Gradient analysis revealed directional recovery from the outfall of the invertebrate community from opportunistic faunal associations (e.g., surface-feeding tubicilous polychaetes) to later successional stage associations of burrowing errant or tube-dwelling "head-down" deposit feeders. Dominant invertebrate fauna which demonstrated effects and recovery due to organic enrichment associated with pulp mill effluent, comprised an association of bivalves, a hydroid, sedentary and errant polychaetes, including: *Axinopsida serricata, Mediomastus ambiseta, Minuspio multibranchiata, Cossura modica, Amphararete acutifrons* and *Pholoe glabra.*

Morphological Deformities of Indicator Benthic Invertebrates as an Assessment Tool in EEM. G.P. Thomas and N. Munteanu. G3 Consulting Ltd., Richmond, BC.

Benthic invertebrates have been useful in environmental monitoring assessments such as EEM given their ubiquity, sedentary behaviour, ease of collection, and, demonstrated tolerances and intolerances to specific contaminants. Use of indigenous benthic communities, as part of Cycle 1, was successful in some instances in demonstrating extent and magnitude of exposure to effluent from pulpmills. This paper discusses how the assessment of benthic morphological deformities may also assist in further defining the magnitude and extent of mill and system associated effects, previously too subtle to distinguish at the community level. Common deformities assessed include numerical asymmetry, bilateral asymmetry and reversion. The merits (and limitations) of utilizing deformity observations to support community structure assessments (e.g., biomass, species abundance and richness) for EEM are discussed. Such assessments represent a logical step in EEM invertebrate community studies. Additional strengths and weaknesses of the technique are also reviewed in this paper.

The Use of TOC, TN (C/N Ratio), TS and Eh for Examining Pulpmill Effluent Impacts on Marine Sediment Quality and Benthic Invertebrate Communities. M. Davies and L. Young. Hatfield Consultants Ltd., West Vancouver, BC.

EEM Cycle 2 for the pulp and paper industry added the following four variables to supporting environmental measurements for assessing marine sediment quality: total nitrogen (TN), carbon/nitrogen ratio (i.e., total organic carbon [TOC]/TN), total sulphides (TS), and Eh (redox potential). C/N ratio provided information on the relative contribution of terrestrial and marine sources of carbon, to aid in defining discharges of terrestrial organic materials (e.g., fibre) in pulpmill effluent into marine receiving environments. Redox potential and total sulphides provided evidence regarding the oxidative state of sediments.

Hatfield Consultants Ltd. conducted marine invertebrate community surveys for five EEM Cycle 2 programs in British Columbia. Sediment was subsampled from each benthic invertebrate grab for measurements of these four sediment quality variables (n=68 stations, 204 samples/variable). Samples were collected using a 31 cm Smith-McIntyre grab. Regression and multivariate analyses were performed to compare these variables and TOC with each other and with benthic invertebrate community data to determine their correlation.

Results of these analyses suggest that except in environments exhibiting extreme degradation (e.g., grossly anoxic sediments), no single sediment quality measure effectively predicts benthic invertebrate community heath or composition. Individual sediment measures often were highly variable among replicates from individual stations, emphasizing the value of within-station replication and the influence of micro-scale patchiness of benthic habitats on environmental monitoring program results and interpretations. Correlation between the various sediment variables, and between individual sediment variables and benthic abundance and diversity, generally was poor.

Results suggest that guideline criteria put forward in the EEM Technical Guidance Document for these sediment measures are not appropriate indicators of degrees of impact of pulpmill effluent on benthic invertebrate communities. However, examined synoptically, each sediment variable contributed valuable information to a weight-of-evidence approach to impact assessment of marine benthic invertebrate communities.

Environmental Effects Monitoring: Mining (I)

The Metal Mining EEM Program - An Overview. K. Hedley[1] and R. Michelutti[2]. [1]Metal Mining EEM Working Group, National EEM Office, Environment Canada, Ottawa, ON; [2]Metal Mining EEM

Working Group, Falconbridge Ltd., Falconbridge, ON.

The multi-stakeholder Metal Mining EEM Working Group recently completed the EEM Requirements for Metal Mining. These Requirements represent consensus of a broad range of stakeholder interests and will form the basis of the Metal Mining EEM program to be incorporated in an amended *Metal Mining Liquid Effluent Regulation* expected to be published in the Canada Gazette in December 1999. The recommended program will require mines to monitor fish, benthic invertebrates, water, sediment, effluent characterization and sublethal toxicity to determine if the mining effluent is having an impact on the aquatic environment. Each monitoring component has been designed to answer key questions regarding the potential impact of the mining effluent on the aquatic ecosystem. One of the key features of the program is that the program is tiered with future monitoring requirements based on previous EEM results. This will lead to more extensive monitoring being conducted where effects have been identified and the flexibility for mines to reduce monitoring programs where there is evidence there are no effects from the mine effluent. The program also allows for mines to use relevant historic monitoring data, or data collected through programs required by other regulatory agencies, based on the ability of this data to answer the key questions. The program will be implemented similar to the Pulp and Paper EEM program. Mines will be required to submit to Environment Canada study designs prior to proposed field work, and interpretive reports after completion of field work. Environment Canada, through the Regional Offices, will form Technical Advisory Panels to review proposed study designs and interpretive reports.

Metal Mining EEM - The Fish Monitoring Program. R. Prairie[1], K.R. Munkittrick[2], A. Rosaasen[3], M. McMaster[4] and S. Ribey[5]. [1]Noranda Technology Centre, Pointe Claire, QC; [2]Environment Canada/University of New Brunswick, Fredricton, NB; [3]Cogema Resources Inc., Saskatoon, SK; [4]Environment Canada, National Water Research Institute, Burlington, ON; [5]Environment Canada, Hull, QC.

As part of the amended *Metal Mining Liquid Effluent Regulations* under the *Fisheries Act*, mines will be required to develop and conduct an Environmental Effects Monitoring (EEM) program. EEM will be done to evaluate the effects of mine effluent on the aquatic environment, specifically fish, fish habitat, and the use of fisheries resources, as defined by the *Fisheries Act*. Therefore, one important element of the mining EEM program is fish monitoring. Mines will be required to determine if there have been changes in the fish community, fish population and usability of fish due to the mine effluent. Fish collected from an area exposed to mine effluent and from a reference area will be compared in order to determine if there is an effect and if the effect is mine related. The fish community survey will identify suitable sentinel species for the fish population survey. The fish population survey will examine growth and reproduction of resident sentinel species. Fish usability will be determined based on the appearance of fish, flavor or odor, and contaminant levels in fish tissue. It is recognized that some mines may not be able to implement a fish monitoring program as outlined, so it has been recommended that alternative methods, such as a caged bivalve or on-site bioassay study, could be used. Subsequent phases of the program will focus on continuing to monitor the fish population and determining the magnitude, extent and cause of effects (if any). Frequency of monitoring will be dependent on previous fish and benthic invertebrate monitoring results.

Metal Mining EEM - The Benthic Invertebrate Monitoring Program. N. Glozier[1], J.M. Culp[1], M. Wiseman[2] and G. Watson[3]. [1]Environment Canada, National Water Research Institute, Saskatoon, SK; [2]Falconbridge Ltd., Timmins, ON; [3]Inco Ltd., Copper Cliff, ON.

Mines will be required to develop and conduct an Environmental Effects Monitoring (EEM) program as part of the amended *Metal Mining Liquid Effluent Regulations* under the *Fisheries Act*. To evaluate the effects of mine effluent on the aquatic environment, an essential component of the MM EEM will be the assessment of fish habitat as measured by the benthic invertebrate community. Mines will be required to determine if there have been any changes in the benthic invertebrate community due to the mine effluent. For the MM EEM benthic invertebrate monitoring programs, study designs have been standardized and those available fall into three basic categories, each with different philosophical approaches. These are: the Control/Impact or Multiple Control/Impact designs which are ANOVA type designs used to determine the magnitude of difference between exposed and reference areas; the Gradient (simple, radial or multiple) designs, which are intended to examine changes in community structure along a physical and/or effluent gradient which are better suited to regression or continuum analyses; and the multivariate approach of the Reference Condition which compares potential "impaired" or test stations to an ellipse of reference stations.

The benthic invertebrate component focuses not only on standardized sampling designs, site definitions and methodology but also on a site-specific, multi-tiered approach to a monitoring program at a given mine site. For example, only after an effect at a given mine site is established, is the extent, magnitude and cause of the effect investigated. An example of this multi-tiered approach is the progressive nature of benthic invertebrate taxonomic resolution. During the first monitoring phase (Initial Monitoring), benthic invertebrate identification is recommended at a higher taxonomic level than later in the program when magnitude and cause of the effect are the primary questions. Furthermore, a progressive addition occurs throughout the program in regards to other biotic indicators within the aquatic food web which may aid in determining the magnitude of observed effects. Finally, integration between the components has occurred as sampling frequency for benthic invertebrates depends not only on the results of the previous benthic invertebrate surveys but on fish survey results and other appropriate information which is concurrently being collected for a particular mine site.

Metal Mining EEM - Effluent Characterization, Water Quality and Sediment Monitoring. R. Parker[1], J. Fyfe[2] and C. Dumaresq[3]. [1]Environment Canada, Fredericton, NB; [2]Falconbridge, Onaping, ON; [3]Environment Canada, Hull, QC.

The amended *Metal Mining Liquid Effluent Regulations* under the *Fisheries Act* will require all regulated base metal and gold mines to monitor fish and benthic invertebrates, and collect supporting environmental information on effluent, water, sediment and effluent sublethal toxicity to evaluate if the mine effluent is having adverse effects on the aquatic environment. Mines will be required to conduct effluent and water quality monitoring a minimum of four times per year to determine the effluent contaminant loadings to the aquatic environment and to measure contaminant concentrations in the study area. Effluent and water samples will be analyzed for the MMLER regulated parameters plus other potential metals and contaminants and for supporting chemical parameters subject to some site specific exemptions. At a minimum, water quality monitoring must be conducted at a reference (uncontaminated) area and an effluent exposed area.

Sediment samples will be collected concurrently with benthic invertebrate sampling. A characterization of sediment will be required to determine if there are habitat differences that may contribute to effects in benthic invertebrates communities. This will involve measurements of particle size distribution and total organic carbon. Chemical analyses of sediment for metal concentrations will be required if an effect is observed on benthic invertebrates. Water and sediment monitoring are essential components for the interpretation of any biological effects observed, to determine if effects are mine related, and to estimate or predict the geographical extent of those effects. A broader range of monitoring tools and methods are suggested for focused monitoring or to determine the cause of the effect. These additional tools could include such things as the measurement of dissolved metals in water, sediment toxicity tests or sediment pore water chemistry.

Recommendations of Mining EEM Toxicology Subgroup and Summary of Proposed Requirements. R. Scroggins. Environment Canada, Gloucester, ON.

A twelve-person multi-stakeholder Subgroup on laboratory toxicology was formed in December 1997 to provide technical advise to the EEM Working Group on the implementation of sublethal toxicity monitoring as a component of the Environmental Effects Monitoring requirements under the amended *Metal Mining Liquid Effluent Regulation.* From January 1998 to April 1999, the Subgroup met on 12 occasions to: assess existing sublethal toxicity data for mine effluent or mine-impacted surface waters; discuss issues related to the use of sublethal toxicity in monitoring metal mining effluents; develop consensus recommendations on EEM sublethal toxicity requirements; and develop technical guidance which supports the implementation of the sublethal toxicity requirements and data interpretation. The presentation will focus on the specific consensus recommendations of the Subgroup and the proposed requirements for sublethal toxicity negotiated by members of the EEM Working Group.

Environmental Effects Monitoring: Mining (II)

In Situ **Periphyton – A Tool Worthy of Consideration in Mining EEMs.** G.P. Thomas and N. Munteanu. G3 Consulting Ltd., Richmond, BC.

A principal challenge facing EEM researchers is demonstrating exposure and delineating extent of discharge plumes. This challenge becomes further compounded by environmental complexity, existing stresses to indigenous biotic communities and fish mobility. Assessment of periphyton communities presents a scientifically-relevant and cost-effective alternative which addresses the objective of defining the extent and magnitude of discharge effects on indigenous communities. This paper describes the application of *in situ* periphyton (attached algae) as an effective, inexpensive tool in environmental assessment. Algal species, comprised of periphyton colonizing artificial surfaces, lend themselves to gradient studies as they are ubiquitous, well studied and understood as contaminant indicators, and, demonstrate known tolerances and intolerances to specific constituents and concentrations of mine effluent. This tool effectively applies the control of mesocosm and caged animal studies without compromising ecological relevance. This paper describes recent innovations applied to periphyton science and its application to EEM. This method is compared with the invertebrate community studies (ICS) and advantages and similarities are delineated, and endpoints compared, including use of morphological deformities as a diagnostic tool. Case studies of periphyton community structure analysis in environmental monitoring of mining impacts are also discussed. Application of this tool to the MM EEM are reviewed.

Metal Mining in British Columbia: Implementation of Environmental Effects Monitoring.
M.E. Hagen[1] and I.D. Sharpe[2]. [1]Environment Canada, North Vancouver, BC; [2]British Columbia Ministry of Environment, Lands and Parks, Smithers, BC.

Environmental monitoring programs, administered by the British Columbia Ministry of Environment, Lands and Parks (MELP) via the provincial *Waste Management Act* (WMA) permit system, are in place for many British Columbia (BC) mines. It will be challenging to coordinate federal metal mining environmental effects monitoring (EEM) requirements under the revised *Fisheries Act Metal Mining Effluent Regulations* with the existing provincial program. This paper presents an overview of the BC approach to EEM, and gives a starting point for integration of the two programs.

Complex Geology
EEM in BC is complicated by complex geology. There is a distinct north-west to south-east tectonic belt structure, paralleling the Rockies and the coast. The former continental margin is at the Rockies. Continental drift, volcanism, glaciation, and other geological processes operating over hundreds of millions of years has resulted in a patchwork of porphyry, sedimentary-exhalative, massive sulphide, mesothermal, epithermal, and skarn deposits. An eclectic combination of Au, Ag, Cu, Pb, Zn, and Mo and other minerals in the ore bodies are mined in BC.

A wide variety of processes are used to extract and concentrate the ore, creating a variety of minewater, site runoff, tailings pond supernatant, water treatment plant effluent, and seep discharges. Effluent is discharged to a variety of environments ranging from high alpine areas to temperate rainforest lowlands. Valued resources in need of protection include fish, fish habitat, other aquatic organisms, waterfowl, wildlife, and human uses of water such as domestic consumption, agriculture, and industrial use. This biological and physical complexity poses challenges for pollution control planning, monitoring, and impact assessment.

BC Ministry of Environment, Lands and Parks Administrative Regions
The BC Ministry of Environment, Lands and Parks implements its pollution prevention provisions via seven administrative regions (Fig. 1). Each region has impact assessment biologists, WMA permit writers, and administrators working to monitor, assess, mitigate, and control discharges from the industries in their region. Regions have a varying number of mines under their jurisdiction (Table 1).

BC Metal Mines
There is a continuum from the discovery of an ore body and the exploration of reserves through development, commercial operation, closure and reclamation, and abandonment of a mine (Fig. 2). It is not always clear where a mine is along this continuum.

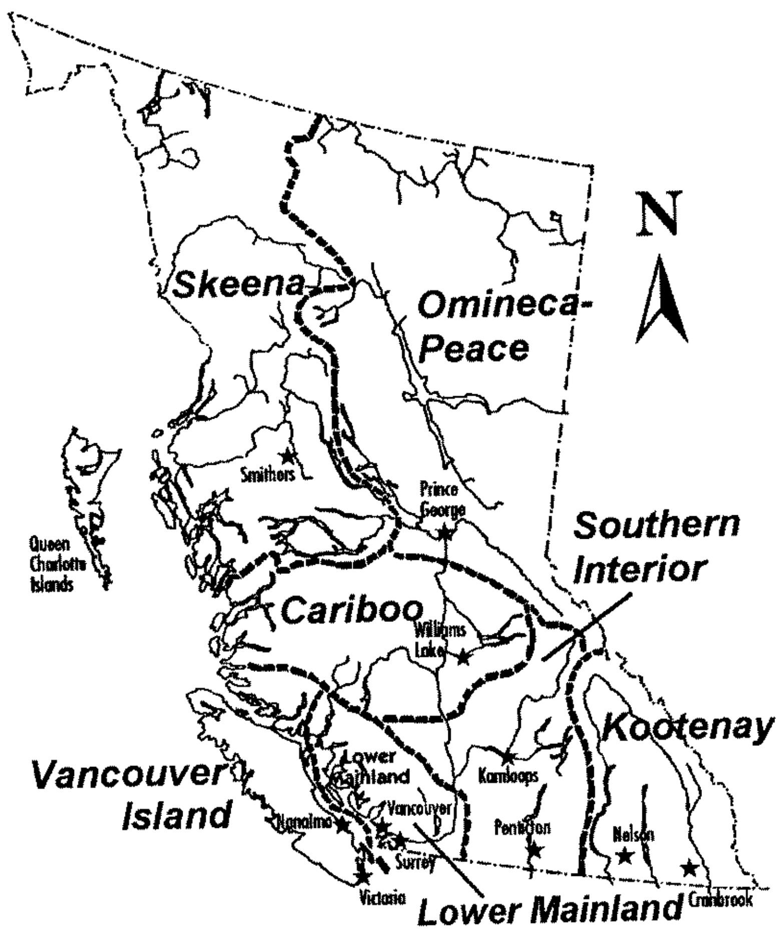


Fig. 1. BC Ministry of Environment, Lands and Parks Regions

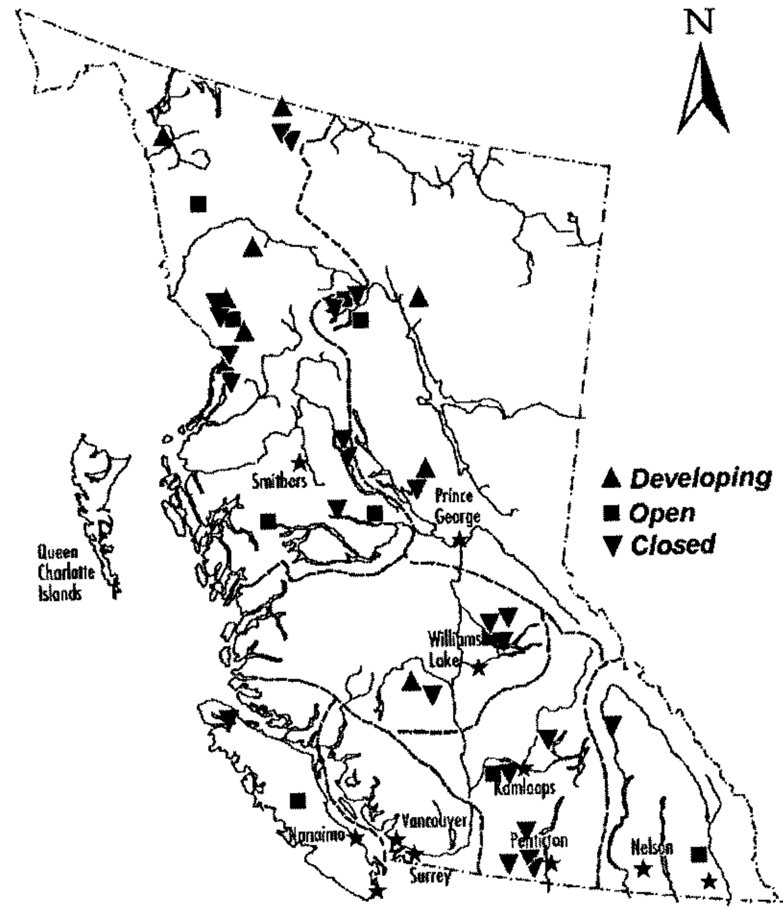


Fig. 2. Mines in British Columbia

After exploration, a company may apply to the BC Environmental Assessment Office for an approval in principle. The Office administers the BC *Environmental Assessment Act* (EEA), and accommodates federal *Canadian Environmental Assessment Act* (CEAA) requirements under a harmonization agreement. If approved, a mine receives a development certificate, and can proceed through permitting phases.

Operating mines require MELP WMA permits, which normally include receiving environment water quality monitoring requirements, and may specify water quality objectives that must be met. Permits may also include biological monitoring of, for example, metal levels in fish tissue, and may include environmental effects monitoring.

BC Ministry of Energy and Mines (MEM) *Mines Act* permits are also required. These specify geotechnical, reclamation, and health and safety requirements. An abandoned mine is one where all permit obligations are satisfied, and mineral rights have reverted to the province. There are approximately 1500 abandoned mines in BC, including coal and industrial minerals.

Exploration: 12,000

	Cariboo	Kootenay	Lower Mainland	Omineca-Peace	Skeena	Southern Interior	Vancouver Island	TOTAL
Developing:	1	0	0	2	6	0	0	9
Open:	2	1	0	2	4	1	1	11
Closed:	3	1	0	3	9	6	1	23
TOTAL:	6	2	0	7	19	7	2	43

Abandoned: 1,500

Table 1. Number of mines in BC

BC Metal Mining Regulation

Pollution Control Objectives for Mining, Smelting and Related Industries of BC were established in the 1970s. These objectives preceded the WMA (1982) and may still be used when establishing permit conditions, although more stringent site specific requirements are the norm. The federal *Fisheries Act Metal Mining Liquid Effluent Regulations* were promulgated in 1977. This 20 yr regulation of mining effluents includes environmental monitoring for water quality. Since the 1980s, biological monitoring (including effects) has been incorporated in WMA permits on a site specific basis.

The BC regulations allow an integrated regulatory system. Baseline inventory and impact/risk assessment occur under the EAA. This feeds into effluent permit conditions under the WMA which also set operating conditions. Reclamation permits under the *Mines Act* set ongoing reclamation requirements and set the stage for long term closure conditions. Throughout this, EEM is used to evaluate and fine-tune permits and provide environmental protection assurance to stakeholders. It could be thought of as a "cradle to grave" environmental monitoring program.

Site Specific EEM Program Development
An iterative design approach is used to design a monitoring program at each site (Fig. 3). This model is flexible to allow integration and consideration of new information and data as it becomes available. The five phases to the systems design approach (analysis, design, development, implementation, and evaluation/revision) are non-linear as the model is interactive, iterative, and evolving. We may predict impacts, demonstrate how hazardous conditions can be identified and mitigated, analyse effects that result and/or are identified in a monitoring program, and feed back to develop and implement mitigation measures and assess their effectiveness.

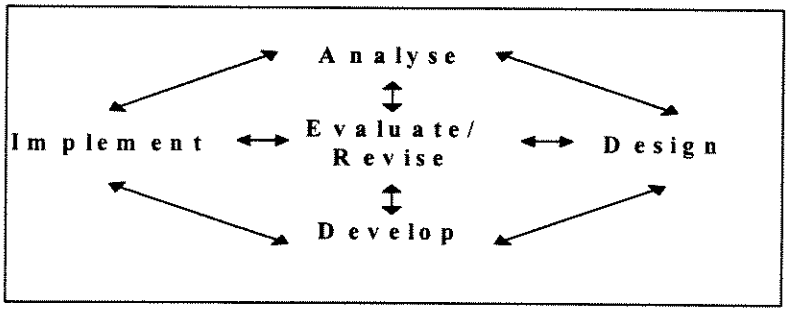


Fig. 3. Iterative design approach

EEM Guiding Principles
A primary guiding principle used to apply the systems design approach is that uncertainty limits tolerance for risk. Risk, itself, varies depending on the quality of the effluent, and the type or amount of valued ecosystem components in the vicinity. This means that as value, uncertainty, and/or risk increase, so does the rigor and/or conservatism demanded in the site-specific EEM program. This affects the type, number, and timing of EEM tools chosen. From this beginning, the monitoring program for a high uncertainty, risk, and/or value area may be pared down as results and circumstances dictate. Alternately, if much is known about the effluent and resources potentially at risk are of low sensitivity to impacts, a stepwise approach to EEM may be used, which employs experimental designs that fit the perceived risk. Integrating EEM information into WMA permits connects effects to pollution control decisions. The need to respond to stakeholder concerns, identify causes, initiate remediation, and assess effectiveness of remediation becomes an explicit part of the permit.

EEM Program Development Process
The development process of an EEM Study Design is coordinated among the stakeholders. While mines are responsible for drafting a Design according to regulatory requirements or terms of reference that are suited to the individual operation and discharges, government agencies and other stakeholders, including First Nations and members of the public, may comment, and MELP may require changes.

The range of resource values present and the knowledge of site-specific constraints is considered first. The appropriate tools from the EEM toolbox are selected. Tools commonly include acute and sublethal toxicity testing; fish health indicators, tissue chemistry, or population surveys; benthic invertebrate or periphyton community surveys; water and sediment chemistry; and hydrology. Other, less common, tools such as invertebrate tissue chemistry, or physiological impact indicators, may also be used.

For a conservative program, all or most of the common monitoring tools are used from the outset, within highly rigorous experimental designs. For lower risk situations, fewer tools are used, and may be employed at a lower frequency, or in a statistical design that reflects a higher tolerance for uncertainty through the choice of effect size and the degree of acceptable statistical error. If the situation allows, focussing on the most sensitive or easily assessed receptor(s) first provides needed environmental protection, at a more reasonable cost. This process leads to explicit waste discharge conditions, including volume and concentration limits within WMA permits, and mine closure conditions in *Mines Act* permits.

EEM is also used to focus compliance monitoring, promotion, and enforcement, and to optimize regulatory approaches to mining. For example, finding a measurable effect in the aquatic environment may lead to a toxicity identification and reduction exercise, or a change in the use of treatment technology as a requirement in the *WMA* permit.

EEM Components for Four BC Mines

Table 2 shows the EEM components and their emphasis for four Skeena region mines. They are ordered by the subjective/qualitative judgement of increasing risk and, hence, conservatism. Golden Bear is a relatively small, seasonal, gold heap leach operation discharging small volumes to a high elevation, low productivity aquatic environment, some distance from fish bearing waters. Hence, end-of-pipe effluent monitoring is emphasized, with relatively little effort placed on environmental effects monitoring.

	Baseline	Water Quality	Sediment	Acute Toxicity	Sublethal Toxicity	Fish	Benthos	Algae
Golden Bear	3 - 1950s	3	2	3	-	Not proximal	3	1
Endako	1 - 1950s	3	2	3	4	1	3	3
Huckle-berry	5 - Late 1990s	5	1	3	-	Benign Discharge		
Eskay Creek	4 - Early 1990s	5	4	4	5	Not present	5	1

1 - low emphasis → 5 - high emphasis, relative to programs at other sites

Table 2. Emphasis on EEM components used at four Skeena region mines

Endako mine has five separate discharges ultimately entering Francois Lake and the Endako River. Data from a 300-yr-long sediment core from Francois Lake showed that lake-wide mining impacts occurred in past decades, but that in the last ten years metal deposition rates have returned to pre-disturbance levels. Tiered benthic invertebrate, periphyton, and sublethal toxicity testing studies showed that impacts to benthic invertebrates occur from the discharges, but are limited to the small receiving streams in the immediate vicinity of the discharges. These areas are not frequented by fish. Means of limiting these minor impacts and monitoring recovery are currently being planned.

Huckleberry mine is a large open pit operation, with three discharges to Tahtsa Reach (part of the Kemano Reservoir). Currently, discharge chemistry is relatively benign, but acid rock drainage (ARD) potential exists at this site. The WMA permit does not require fish, benthos, or algae programs because the risk associated with each of the discharges is low given their contaminant loadings.

Reconnaissance level receiving environment sediment sampling occurred in 1998 to validate the decision to hold biological EEM tools in abeyance for now at the Huckleberry mine. In addition to discharge chemistry monitoring, groundwater chemistry near the property boundary and water quality monitoring in Tahtsa Reach opposite one of the discharges is required in the permit. This latter requirement is in response to an issue regarding nitrite in discharged mine pit water, while groundwater monitoring is for early warning of ARD. Acute toxicity tests are required twice a year for Huckleberry's discharges, to provide a warning increases in loadings of contaminants which may not be caught by the water quality program.

Unlike Huckleberry, the Eskay Creek mine discharges to an alpine area devoid of fish and of low productivity. But Eskay Creek discharges tailings to an unconfined alpine lake, the only mine in Canada to do so. Due to the nature of the discharge chemistry (bio-accumulative metals, among a large suite of metals), and unique receiving environment (discharges from the tailings lake enter a highly variable, glacially fed creek), a high degree of emphasis is placed on biological monitoring at Eskay Creek. Much of the focus is on the "sediment triad approach" (benthic invertebrate populations, sediment chemistry, and sediment toxicity) as an indicator of metals and other mining related contaminant impacts, and caged mussels as an indicator of mercury bio-accumulative impacts. Sublethal toxicity testing of effluents also figures prominently in the EEM program.

As illustrated by these four examples, a wide variety of conditions and environmental protection circumstances in BC result in a range of environmental effects monitoring approaches and programs. As will always be the case, the challenge is to continue to improve our understanding of mining impacts regardless of federal or provincial involvement, and to provide the public with assurances that our precious aquatic resources are afforded a high degree of protection.

Coordination Strategy

Federally, EEM is coordinated by the National EEM Office in Ottawa. In the pulp and paper sector, a Management Steering Group and a Technical Management Committee have representation from both government and industry. Similar groups may be established for the mining sector.

The federal program is delivered by Regional Coordinators and Authorization Officers in each of the five Environment Canada regions. The BC provincial program is delivered autonomously in each BC MELP region. There is no equivalent provincial EEM coordinating office in Victoria, although Water Management Branch and Laboratory Services specialists provide advice upon request.

Local Monitoring Committees

In the federal EEM Pulp and Paper program, Technical Advisory Panels (TAP) are established for each province except BC. TAPs review and comment on the EEM Design and Interpretive Reports for the mills in their province. In BC, Local Monitoring Committees (LMC) are instead established for each mill, and include representatives from Environment Canada, Department of Fisheries and Oceans, BC Environment, the mill, and the mill's consultant. Representatives of environmental organizations and/or First Nations may also participate on TAPs or LMCs.

This cooperative approach has worked successfully in BC, and good working relationships between government agencies and industry are the result. This model could provide the needed liaison between the provincial and federal mining EEM systems.

Local Monitoring Committees for mining EEM programs in British Columbia will likely be chaired by the MELP regional environmental officers given the provincial experience with mining environmental effects monitoring in BC.

It is hoped we can combine the strengths of both provincial and federal EEM programs into one for BC. This includes using provincial strengths such as local knowledge and expertise to respond to site-specific conditions and constraints, and federal strengths such as nationally consistent standards, including QA/QC standards, and the ability to apply national resources and experiences to resolution of site-specific issues.

Further information:
Information sessions are planned for each province during the two-month consultation phase following Gazette 1. Program details are available, or will be available over the next few months, on the Environment Canada Green Lane (National EEM Office: <http://www.ec.gc.ca/eem/english/default.htm>; Pacific Region EEM Program: <http://www.pyr.ec.gc.ca/ep/eem/eem.html>).

See also:
BC Ministry of Environment, Lands and Parks (http://www.env.gov.bc.ca/)
BC Ministry of Energy and Mines (http://www.em.gov.bc.ca/)
BC Environmental Assessment Office (http://www.eao.gov.bc.ca/)

Oxyradical and Toxicological Potential of Leachate from Iron-ore Mines in Labrador. D. Hamoutene[1], A. Rahimtul[1] and J. Payne[2]. [1]Biochemistry Department, Memorial University, St John's, NF; [2]Department of Fisheries and Oceans, Science Branch, St John's, NF.

The ecotoxicological effects of mining effluents is coming under much greater scrutiny in Canada. It appears necessary to explore possible health effects in association with iron-ore mining effluents. Our results clearly show that iron-ore leachate is not an inert media but has the potential to induce lipid peroxidation. Peroxidation was assessed by measuring oxygen consumption in presence of a reducing agent such as ascorbate or NADPH and chelators such as EDTA. The Labrador iron-ore is an insoluble complex crystaline material containing a mixture of metals (Fe, Al, Ti, Mn, Mg, etc) in contrast to the iron sources used for normal lipid peroxidation studies. The metal of highest percentage is iron (59.58%), a metal known to redox cycle and induce oxyradical production. Iron-ore powder initiated ascorbic acid-dependent lipid peroxidation (nonenzymatic) in liposomes, lipids extracted from rat and salmon liver microsomes and intact salmon liver microsomes. It also showed an inhibitory effect on NADPH-dependent microsomes lipid peroxidation as well as on NADPH cytochrome c reductase activity. However, no effect was observed on cytochrome P450 IA1 and IIB1 dependent enzymatic activities and on

P450 levels. This inhibition could be due to one of the other components of iron-ore leachate (Mn, Al, etc). Nonenzymatic lipid peroxidation in rat liver microsomes was not significantly inhibited. These conjugated effects of iron-ore leachate clearly indicate that a potential toxicity could be associated with its release in Canadian lakes. Further studies are necessary to explore *in vivo* effects on aquatic animals knowing that iron-ore has the ability to produce redox active and thus potentially damaging oxidative species. (Supported by Toxic Chemicals Program, Department of Fisheries and Oceans.)

Investigations of Dietary Arsenic Exposure in Lake Whitefish (*Coregonus clupeaformis*)**: Accumulation, Distribution, and Initial Findings on Toxicological Effects.** R.M. Pedlar[1] and J.F. Klaverkamp[1,2]. [1]Department of Zoology, The University of Manitoba, Winnipeg, MB; [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

The discharge of effluents into receiving waters by gold and uranium mining and milling operations has resulted in high concentrations of As in sediments and benthic invertebrates. As is available to fish living in effluent receiving waters through consumption of As in contaminated food organisms and sediments. Due to the need for dietary As research, adult lake whitefish were fed As contaminated diets at nominal concentrations of 0, 1, 10, and 100 mg As/kg diet (d.w.) for 10, 30, and 64 d. Liver, kidney, stomach, intestine, pyloric caeca, gallbladder, skin and scales were analyzed for As content. The pattern of As accumulation in fish tissues was influenced by feed refusal beginning on day 45 by fish fed the 100 mg As/kg food. Significant As accumulation occurred in all tissues examined from fish exposed to the 100 μg As/g food for 30 d, with the exception of gallbladder. The highest concentration of As occurred after 30 d of exposure, in pyloric caeca of fish fed the 100 mg As/kg food. Significant accumulation of As occurred in livers and scales of fish fed concentrations of As as low as 10 mg/kg for 30 and 64 d. Analyses of As in pyloric caeca, liver and scales are recommended to evaluate the bioavailability of As to fish in metal mining EEM programs. The toxicological effects of As were assessed at the biochemical level (lipid peroxide production and metallothionein (MT) induction), tissue and organ level (blood parameters, LSI, and histopathology), and at the individual level (morphometric indices). Significant increases in MT concentrations were observed in livers of fish fed the 100 mg As/kg food for 10 and 30 d. At day 64, MT concentrations significantly increased in livers of fish fed the 1 and 10 mg As/kg food. There was a decrease in liver somatic index of fish fed the 100 mg As/kg food for 64 d. Focal necrotic lesions were also observed in livers of exposed fish. MT induction in liver tissue and histopathological lesions may be useful early warning indicators of As exposure when assessing fish health during metal mining EEM programs.

Dietary Exposure of Lake Whitefish (*Coregonus clupeaformis*) **to Nickel: Accumulation, Distribution and Toxicology.** M.D. Ptashynski[1] and J.F. Klaverkamp[1,2]. [1]Department of Zoology, University of Manitoba, Winnipeg, MB; [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

Base metal, uranium, and gold mining and milling activities release significant amounts of Ni into the aquatic environment. In Ni-contaminated systems benthic-feeding fish are exposed to Ni through the ingestion of invertebrates and contaminated sediments. A laboratory-based exposure of fish to Ni, through the dietary route of uptake, was conducted to provide data most relevant to

the chronic exposures that are occurring in the environment.

Lake whitefish (*Coregonus clupeaformis*) were fed control and Ni-contaminated diets (10, 100, and 1000 mg Ni/kg) for 10, 31 and 104 d. Kidney, liver, stomach, intestine, gill, pyloric caeca, skin, and scales were analyzed to evaluate the accumulation and distribution of Ni. Ni accumulation was greatest in the intestine and pyloric caeca of fish fed the high dose diet for 10 d. Accumulation in the pyloric caeca and intestine was dose-dependent. Regulation of Ni through increased excretion and/or controlled uptake may explain the decreased Ni absorption observed after 104 d of exposure. Ni accumulation, in the remaining tissues, was dose and duration-dependent, with the highest concentrations observed in the kidney and scales. Fish fed the medium and high dose diets accumulated significant amounts of Ni in a majority of the tissues sampled, even after 10 d of exposure. The tissues that best assess Ni bioavailability are the kidney and scales. The toxicology associated with the exposure was investigated at the biochemical (metallothionein induction and lipid peroxide production), cellular (histopathology), and organ and whole organism level (condition factor, growth, GSI, and LSI). A linear relationship was observed between Cu and metallothionein concentrations in the liver.

Results indicate Ni was affecting Cu metabolism in the liver. Significant increases in lipid peroxide production were observed in plasma of whitefish fed 1000 mg Ni/kg for 31 d, indicating oxidative stress was occurring. Organ and whole organism indices were not affected by the exposure. A histopathological evaluation is currently being conducted. A qualitative assessment of the liver indicates that increased focal necrosis was observed in fish fed 1000 mg Ni/kg for all durations. The preliminary evaluation of the toxicology associated with the exposure indicates that these cellular and biochemical markers warrant consideration as early warning indicators of Ni exposure. The results obtained from this research, assessing Ni bioavailability and the effects of Ni on fish health, can be used to guide field bio-monitoring efforts in EEM programs.

Environmental Effects Monitoring: Mining (III)

Qualifying the Protective Effects of Dissolved Organic Matter Against the Physiological and Toxicological Effects of Metals in Freshwater Fish. J. Richards[1], M.L. Schwartz[1], J. Curtis[3], K Burnison[4] and R. Playle[2]. [1]Department of Biology, McMaster University, Hamilton, ON; [2]Department of Biology, Wilfrid Laurier University, Waterloo, ON; [3]Faculty of Science, Okanagan University College, Kelowna, BC; [4]Environment Canada, National Water Research Institute, Burlington, ON.

Adult rainbow trout were exposed in ion-poor water to 0.2 µM Cd and 0.8 µM Cu in the presence and absence of dissolved organic matter (DOM). The trout were cannulated for repetitive blood sampling. Natural DOM (8 mg C/L) and Aldrich humic acid (7 or 22 mg C/L) prevented Cd and Cu accumulation in fish plasma, and protected against the ionoregulatory and respiratory disturbances of Cd and Cu. There were no adverse effects of 31 mg C/L DOM alone. We and others have evidence that DOM quality, as well as quantity, influences the extent of the protective effects of DOM. For example, the source of DOM affected the amount of Hg and Pb binding to trout gills and affected the toxicity of a mixed metal exposure. DOM quality will influence how the developing metal-gill or "biotic ligand" models for metal toxicity are applied in future. Characterization of DOM can be relatively complex and expensive (e.g., ^{13}C NMR) or relatively simple and cheap (e.g., specific absorption coefficient). We are investigating methods to identify simply the chemical properties of DOM which best correlate with DOM's biologically important protective effects.

Silver Uptake and Depuration in Fed and Unfed Freshwater Rainbow Trout (*Oncorhynchus mykiss*) **and in Fed and Unfed Trout in the Presence and Absence of Complexing Agents.** B. Bertram and R.C. Playle. Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Rainbow trout (~50 g) exposed to -0.1 µM silver as $AgNO_3$ in ion poor water quickly accumulated Ag in their gills, plasma, and livers but showed no ionoregulatory, respiratory or toxic responses over one wk. In contrast, ~50 g trout exposed to -0.1 µM Ag plus a strong complexing ligand, thiosulphate (-5 µM $Na_2S_2O_3$), had slight protective effects against Ag accumulation on the gill but not against Ag accumulation in the plasma or liver compartments. During depuration the presence of waterborne thiosulphate increased the removal of accumulated Ag from the plasma but not from the gills or from the liver. These results indicate that waterborne thiosulphate can act as a partial, additional sink for Ag release from the fish. Food ingestion at 2% body weight per day did not alter Ag entry into the fish. However, we do not yet know whether feeding alters Ag depuration from the fish through the biliary/ faecal route, but we are currently investigating this possibility.

Toxicity Testing of Mine Effluent and Contaminated Surface Water using Simulated Site Water for Dilution and Associated Effects on Bioavailability of Metals. J.E. Schroeder[1], M. Rinker[1], R.C. Playle[2] and D.G. Dixon[3]. [1]Beak International Inc., Brampton, ON; [2]Department of Biology, Wilfrid Laurier University, Waterloo, ON; [3]Department of Biology, University of Waterloo, Waterloo, ON.

Aquatic toxicity tests conducted using metal salts have shown that toxicity is influenced by various physical/chemical characteristics of water, such as hardness, alkalinity, pH and organic matter. These findings have been used to promote the use of uncontaminated water, collected from the vicinity of a discharge, as dilution water in aquatic toxicity tests when predicting environmental effects of whole effluents on the receiving environment. We investigated the effects of dilution water on the expression of toxicity in mining effluents and contaminated surface waters and assessed the use of simulated laboratory water as a surrogate for site water. Sublethal toxicity tests with *Ceriodaphnia dubia* and fathead minnows were performed using three different effluents and two different contaminated surface waters from four Canadian mine sites. Each test was conducted using three different dilution waters: upstream site water; laboratory water; and laboratory water adjusted to the hardness, alkalinity and pH of the site water. Adjustment of laboratory water was accomplished by dilution with deionized water to the desired hardness, which caused a corresponding shift in alkalinity and pH. Chemistry data from toxicity test exposures were compared using geochemical modelling programs such as MINTEQ and PHREEQC to indicate the likely metal species in each dilution water/effluent mixture and to explain differences in toxicity among the different dilution water tests.

Duration of Post-impoundment Increases in Fish Mercury Levels at the La Grande Complex, Québec, Canada. R. Schetagne. Hydro-Québec, Montréal, QC.

At the La Grande hydroelectric complex, Hg concentrations were measured in over 25,000 fish from more than 200 sampling stations located in natural and modified aquatic systems as part of an ongoing environmental effects monitoring program. Five fish species were monitored over a 20 yr period: the non-piscivorous lake whitefish (*Coregonus clupeaformis*) and longnose sucker (*Catostomus catostomus*) and the piscivorous northern pike (*Esox lucius*), walleye (*Stizostedion vitreum*) and lake trout (*Salvelinus namaycush*). Total Hg concentrations were measured by standard cold vapour atomic absorption spectrophotometry. In reservoirs, concentrations in all species increased rapidly after impoundment, peaking after 5-9 yrs in non-piscivorous fishes, and after 10-13 yrs in piscivorous species, then significantly and gradually declined. Peak concentrations reached levels 3-7 times those measured in surrounding natural lakes. Concentrations in the non-piscivorous species have returned to levels typical of natural lakes 10-19 yrs after flooding. In the piscivorous northern pike and walleye the rate of decline, which begins after 15 yrs, strongly suggests that natural concentrations are reached between 20 to 30 yrs after flooding. Results from other reservoirs in Canada and Finland corroborating this duration are also presented. Monitoring of Hg levels in fish and studies of drifting organisms also show that Hg is exported downstream from reservoirs. Data show that, along a series of large reservoirs emptying one into the another, the effect of this export on fish Hg levels is limited to the first downstream reservoir.

Mine Water Discharge and the Effects of Elevated Nickel on the Early Life Stage of Native Fish Species. K.T. Himbeault[1], J.D. Embury[1], L. Adrian[2] and J. Jarrell[2]. [1]Conor Pacific Environmental Technologies Inc., Saskatoon, SK; [2]Cameco Corp., Saskatoon, SK.

Continual increases in Ni concentrations from dewatering discharge resulted in an assessment of the environmental impacts on aquatic life within the receiving waters. An evaluation on the potential impacts to native fish species included an assessment on the development of longnose sucker embryos and larvae exposed to water collected along a spatial gradient within the drainage. Natural waters were used to provide an assessment of site-specific conditions. Toxicity assessment end-points included hatching success, larval survival and developmental abnormalities. Overall hatching success in all treatments was greater than 80% and averaged 90% in both the control and exposure groups. Significant differences in larval survival relative to control waters were evident. The control groups had a >90% larval survival, whereas exposure groups at the highest Ni concentrations had a <5% larval survival. The highest mortality occurred within the first 10 d of post hatching exposure. After the initial mortality, the survival of larvae within the treatments was maintained for the remainder of the test period. These results suggest that native fish species that survive the initial exposure at very early life stages or that hatch in unexposed areas are capable of surviving in the waters receiving mine water discharges with elevated Ni concentrations.

Environmental Effects Monitoring: Mining (IV)

Assessing the Ability Oil Sands Based Wetlands to Support Sustainable Waterfowl Populations. K.E. Bennett and L. Bendell-Young. Department of Biological Sciences, Simon Fraser University, Burnaby, BC.

Juvenile captive mallards (*Anas platyrhynchos*) were held *in situ* on both a reference wetland and an industrially formed wetland. Growth measurements were taken following 0, 2, 5, 9, 13, 19, 25, and 33 d of exposure. Female mallards on the industrial site showed decreased body mass on days 2, 5, 9, and 13. Subsequent measures of female body mass showed no significant site effect. The body mass of male mallards showed a site effect only at days 2, 9, and 13. These findings suggest that growth, and possibly viability, of juvenile waterfowl on oil sands based wetlands may be compromised.

Status of Acute Lethality of Effluents in Canadian Mining Industry. D. Rodrigue[1], S. Andrews[2], P. Rochon[3] and R. Scroggins[1]. [1]Environment Canada, Gloucester, ON; [2]Andrews Environmental, St. John's, NF; [3]Environment Canada, Hull, QC.

In 1998, Environment Canada conducted a review of the acute lethality of effluents discharged from mining operations in Canada. Effluent acute lethality and chemistry data were solicited and summarized to provide an indication of the effectiveness of the *Metal Mining Liquid Effluent Regulations* (MMLER's) to reduce or eliminate effluent acute lethality. Data were solicited from Canadian mine operations directly as well as federal and provincial government staff from 1994 to 1998. This review includes all metal mines (e.g., base metal, precious metal, uranium, iron ore) plus refineries and smelters associated with mine operations. The national coverage is 83% of all active, suspended and closed mine facilities. For active or suspended facilities, there is an overall test pass rate of 83% and 79% for rainbow trout and *Daphnia magna* respectively. The report also compares the acute lethality pass/fail status to the effluent treatment in place at each active or suspended mining facility. More detailed data analyses from the 1998 survey will be highlighted in the presentation.

Can Aquatic Toxicity Test Data with Effluents be Correlated with Receiving Water Effects Data in Canadian EEM Programs? A Plea for Common Sense. G. Gilron[1] and D. Riehm[2]. [1]ESG International, Guelph, ON; [2]Teck Corporation, Vancouver, BC.

Environmental Effects Monitoring (EEM) programs are currently being implemented in Canada for the Pulp and Paper sector (now in Cycle 2), and are now being planned and designed for the Metal Mining sector. An important challenge for these EEM studies is to define what constitutes a "significant environmental effect", based on standard environmental monitoring parameters. Two of the major data elements used to determine effects in a weight-of-evidence evaluation of EEM data are: toxicity test data on final effluents discharged to the receiving environment (i.e., using sublethal chronic tests on fish, invertebrates and plants); and, data on biotic communities in the receiving environment (i.e., fish and benthic invertebrate community surveys). Recently, some innovative studies merging the "lab" and "field" approaches have been conducted (e.g., *in situ* clam bioassays, mesocosm studies). However, these *in situ* approaches have not yet been adopted fully into the EEM approach. Although previous researchers have warned about the dangers of trying to correlate lab toxicity data on effluents with impacts observed in the field (e.g., Underwood, 1995), there is still a lab-field correlation hypothesis, which asserts that these two data elements can somehow be correlated, so that one element can act as a surrogate to the other. In this presentation, we operationally define the endpoints of laboratory-generated vs field-generated data in support of EEM programs. Using these definitions and some recent studies reported in the literature, that have used these two data elements in an integrated fashion, we will attempt to dispel the lab-field correlation hypothesis.

Effects of Metal Mining and Milling on Boundary Waters of Yellowstone National Park. R. Nimmo Delwayne[1] and J.C. Greene[2]. [1]U.S. Geological Survey (retired), Biological Resources Division; [2]U.S. Environmental Protection Agency.

Aquatic resources in Soda Butte Creek within Yellowstone National Park, USA, continue to be threatened by heavy metals from historical mining and milling activities that occurred upstream of the park's boundary. This includes the residue of Au, Ag, and Cu ore mining and processing in the early 1900s near Cooke City, Montana, just downstream of the creek's headwaters. Toxicity tests using surrogate test species, and analyses of metals in water, sediment, and macroinvertebrate tissue were conducted from 1993 to 1995. Chronic toxicity to test species was greater in the spring than the fall and metal concentrations were elevated in the spring with Cu exceeding water quality criteria in 1995. Tests with amphipods using pore water and whole sediment from the creek and Cu concentrations in the tissue of macroinvertebrates and fish also suggest that Cu is the metal of concern in the watershed. In order to understand the current conditions in Soda Butte Creek, heavy metals, especially Cu, must be considered important factors in the aquatic and riparian ecosystems within and along the creek extending into Yellowstone National Park.

Sediment Toxicity (I)

Taking it to the Creek - Using an Organic Pollution Gradient to Evaluate Techniques for Dredged Sediment Assessment. L.M. Porebski[1], B.A. Zajdlik[2], K.G. Doe[3] and J.M. Osborne[1]. [1]Environment Canada, Marine Environment Division, Ottawa, ON; [2]B. Zajdlik & Associates, Rockwood, ON; [3]Environment Canada, Toxicology Laboratory, Moncton, NB.

A pollution gradient of PAHs, PCBs and heavy metals from the mouth of Mugga Creek to Sydney Harbour, Nova Scotia was used to evaluate techniques for the assessment of dredged sediments. Non-contaminant factors at these stations, including depth, grain size and TOC were intended to be as similar as possible to focus the study on contaminant responses. Stations were assessed by a triad of biological testing, sediment chemistry, and benthic community structure analyses. Relationships among these assessment tools were examined. Amphipod survival, solid phase-Microtox® and bioaccumulation assays generally responded more strongly at more contaminated stations. Fertilization success using echinoids and growth using polychaetes however, appeared to be better correlated with other non-contaminant factors. Test variability and interpretation criteria were examined for adequacy. Canadian draft Interim Sediment Quality Guidelines, were used as the chemical benchmarks to select test stations based on the relative probability of effects. Guidelines at the Threshold effects level (TEL) performed well in the study as levels below which unacceptable biological effects were unlikely to occur. Relationships between the three elements of the triad were found using a variety of statistical tools, but could not be confirmed using Mantel's test.

Use of Sediment PCB Contamination, Sediment Toxicity, and Benthic Invertebrate Community Characteristics at the Site of the Irving Whale, in Making Decisions on Remediation. W.R. Ernst, K. Tay, K. Doe, G.R. Julien and P. Jackman. Environment Canada, Atlantic Region, Dartmouth, NS.

Immediately after raising the Irving Whale, a sunken barge which contained PCBs in a heat transfer system, it was determined that sediments in the vicinity were heavily contaminated with PCBs. Removal of the most heavily contaminated sediments occurred within several days of the lift, however a decision on additional sediment remediation was deferred until the extent and risk of the sediment contamination could be better determined. Sediment samples were collected over a three yr period and the rate of dispersion was determined. In addition, toxicity testing using the amphipod, *Amphiporeia virginiana* and the bacterium *Vibrio fischeri*, and benthic invertebrate community sampling were also conducted in order to determine the risk of sediment contamination. Even though relatively high concentration of PCBs (in excess of 1000 mg/kg) remained after three yrs, the toxicity testing and benthic invertebrate community sampling indicated a relatively low level of risk and a decision was made not to conduct further remediation.

Bioavailability and Accumulation of Metals from Stormwater Pond Sediments. T. Hackbarth[1], B.C. Anderson[1], P.V. Hodson[1], W.E. Watt[1] and J. Marsalek[2]. [1]Queen's University, Kingston, ON; [2]Environment Canada, National Water Research Institute, Burlington, ON.

Urban runoff is a major source of non-point pollution to aquatic ecosystems. Stormwater detention ponds or wetlands built to treat this runoff can be quite successful at improving water quality before its release back to the environment. Over time, these ponds usually become habitats for waterfowl, fish, and other organisms, but their the role as reservoirs for a variety of contaminants may pose a risk to the health of the pond ecosystem. We investigated the potential for bioavailability of contaminants from stormwater pond sediments and their accumulation in aquatic organisms as a first step in assessing toxicity of stormwater pond sediments. Freshwater mussels were caged in several stormwater ponds in Kingston and Ottawa, Ontario in which sediment metal concentrations and speciation were known. Metal concentrations were often above the Provincial Sediment Quality Guideline lowest effect levels, suggesting the potential for negative effects on aquatic organisms. Mussels were sampled periodically over 14 wks and their soft tissues analyzed for Pb, Cu, Ni, Cd, and Cr. There was no accumulation of Cd, Cu, and Cr relative to the background concentrations, but an increase in Pb and a decrease in Ni. This suggests that metals found in these ponds, with the exception of Pb, may not enter aquatic food webs. Compared to other studies of metal accumulation in mussels, bioavailability of metals appears limited, perhaps due to relatively low metal concentrations in these sediments.

Apples with Apples: Application of a Rapid Sublethal Sediment Toxicity Test to Assess Recovery of Coastal Marine Fish Habitat. E.R. McGreer. ERM Environmental Consulting, Vancouver, BC.

A rapid, sublethal marine sediment toxicity test using the burrowing rate of the bivalve clam *Macoma balthica* was used to monitor recovery of sediments previously impacted by a major metropolitan sewage effluent discharge. From 1963-1988, the Iona Island Wastewater Treatment Plant (WWTP) discharged up to 90 million gallons per day of primary-treated sewage effluent into a shallow, intertidal area of the Fraser River Estuary, BC. Previous studies had documented a number of biological impacts including sediment toxicity, changes in invertebrate community structure, and fish kills from low levels of dissolved oxygen. In 1988, the original effluent discharge ceased after installation of a 5 km long deep water diffuser. Changes in sediment toxicity pre- and post-discharge were used to evaluate biological recovery of the area. Changes in sediment chemistry showed decreased concentrations of contaminants (e.g., Cu 12%, Pb 41%, and Hg 56%) since the discharge ceased. Sediment PAHs were found to be within Canadian guidelines on all occasions. Burrowing rates for *M. balthica* ranged from 0.5 h for reference sediments to over 4.5 h for the most contaminated sites. Post-discharge monitoring showed that burrowing rates had returned to normal after 8 years at most sites formerly affected by the sewage effluent. The study concluded that there had been an overall improvement in fish habitat since cessation of the sewage discharge. Vital to the success of this effort was the existence of historic information on sediment toxicity collected using standard test methodology.

Bioavailability and Toxicity of Nickel in Sediments to *Hyalella azteca*. W.P. Norwood, U. Borgmann and R. Neron. Environment Canada, National Water Research Institute, Burlington, ON.

The effect of sediment source on chronic toxicity and bioavailability of nickel in sediments to *Hyalella azteca* was investigated. Sediments collected from three sites (Wasaga Beach, Long Point Lake Erie, and Severn Sound, Ontario) were spiked with a range of Ni concentrations. Each sediment had very different properties. Sediment collected off Wasaga Beach was coarse grained with low organic C, the Long Point sediment was fine grained with low organic C, and Severn Sound sediment was fine grained with high organic C. In addition, toxicity tests were performed using two different water to sediment ratios (4:1 and 67:1) resulting in different degrees of leaching of metal and organic matter into the overlying water. Toxicity expressed on a sediment or water concentration basis was highly variable. When expressed relative to the amount of metal accumulated in the body, Ni toxicity was much more constant. Body concentration measurements therefore result in a more reliable prediction of toxic effects than do measurements of Ni in water or sediment.

Sediment Toxicity (II)

Chronic Effects of Tributyltin on the Freshwater Amphipod, *Hyalella azteca*. A.J. Bartlett[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.

Tributyltin (TBT) is used in marine antifouling paint to prevent the attachment of organisms to the hulls of ships. Despite regulations controlling its use, TBT remains an environmental concern because of its persistence in sediments, its toxicity to nontarget organisms, and its continued use. Research has focused on the impact of TBT in the marine environment due to widespread effects in marine invertebrates at extremely low concentrations. Freshwater organisms appear to be less sensitive to TBT, however this may be due to the lack of research on the effects of TBT in freshwater environments. Chronic spiked-sediment experiments were carried out using the freshwater amphipod, *Hyalella azteca*. Amphipods were exposed to a concentration series of TBT-spiked sediments over 20 wks, spanning two generations of animals. Test containers were changed regularly to allow the monitoring of survival, growth, and fecundity during the experiment. Water, sediment, and tissue concentrations were measured, and toxicity relationships were determined for survival, growth, and fecundity. The survival of males versus females was compared. The effects on first generation amphipods were compared to second generation animals. Studies to further characterize the effect of TBT on *H. azteca* are currently underway.

Confounding Factors Reference Values for the 10-day Amphipod Sediment Acute Lethality Test. K-L. Tay[1], K.G. Doe[2], P. Jackman[2] and A. MacDonald[1]. [1]Environment Canada, EPB, Dartmouth, NS; [2]Environment Canada, ECB, Moncton, NB.

Environment Canada, Atlantic Region, has conducted a series of laboratory experiments to generate reference values for confounding factors that influence the results of the 10 d amphipod sediment acute lethality test. Confounding factors investigated included grain size effects, ammonia, and the influence of organic carbon on PAH toxicity. Less than 60% of *Corophium volutator* and *Eohaustorius washingtonianus* survived the artificial mixtures containing 30 to 40% clay. The survival of three other species of amphipods, *E. estuarius, Leptocheirus plumulosus* and *Rhepoxynius abronius,* was greater than 60% in all artificial mixtures (2-99% sand). The undissociated porewater ammonia LC_{50} for the four species of amphipods: *Amphiporeia virginiana, E. estuarius, E. washingtonianus,* and *R. abronius*, ranged from 0.14 mg/L NH_3-N (*A. Virginiana)* to 1.55 mg/L NH_3-N (*E. Washingtonianus).* The marine sediment reference material LC_{50} for the four species of amphipods was between 0.09 mg/kg total PAHs (*E. washingtonianus*) and 4.72 mg/kg total PAHs (*R. abronius*). When 1% humic acid was added to the artificial sediments, the LC_{50} for *A. virginiana* increased from 1.50 to 7.23 mg/kg total PAHs. The results, including NOECs and LOECs for ammonia and PAHs and grain-size tolerance were used to assist in the development of Environment Canada's Reference Method for measuring sediment toxicity using the estuarine/marine amphipods.

Sediment Quality Monitoring at Chrystina Lake near the Swan Hills Treatment Center. C.T. Oishi[1], D.J. Patan[1], M.A. Kavanagh[2] and G.P. Latonas[3]. [1]Komex International Ltd., Calgary, AB; [2]Alberta Energy and Utilities Board, Calgary, AB; [3]Bovar Waste Management, Calgary, AB.

Sediment quality monitoring has been conducted on an annual basis around the Swan Hills Treatment Center since the facility was commissioned in 1987. A comprehensive suite of inorganic and organic parameters have been analyzed including PCBs, and dioxins and furans. Chrystina Lake, located 1.5 km NE of the facility, is one of several sediment monitoring sites in the program. In recent years, attempts to improve the monitoring program have resulted in changes to sampling protocol and analytical procedures. A combination of improved sediment sampling techniques, introduction of sediment core dating, updated laboratory chemical analysis and augmented quality control measures have led to a better understanding of input and increased confidence when interpreting results. This improved program has been used to more accurately determine the levels at which organic anthropogenic chemicals are being deposited into Chrystina Lake. As well, the program has greater capability to determine multiple sources of input. The results of the monitoring program will be presented in relation to previous years results. In addition, a discussion of the significance of the new results will place an emphasis on the importance of quality control measures and how to better interpret the data.

Subsampling in Sediment Quality Assessments: Why Bother? M.D. Paine[1], P.J. Allard[2] and G.A. Mann[2]. [1]Paine, Ledge and Associates (PLA), North Vancouver, BC; [2]EVS Environment Consultants, North Vancouver, BC.

In sediment quality assessments, subsamples or secondary-stage replicates within primary-stage units are often collected or analyzed. For example, field sediment samples are often split into five laboratory replicates for toxicity testing, as specified in most protocols. If the field samples are primary-stage replicates (e.g., from different stations or from different grabs within stations), collecting or analyzing multiple secondary-stage replicates is rarely cost-effective. The statistical power and robustness of ANOVA, regression and correlation analyses depend mostly on the number of primary-stage replicates, until at least 10 are sampled. Strategies and "rules of thumb" for optimizing the allocation of costs and sampling effort between primary- versus secondary-stage replicates will be discussed, and illustrated by example. In most sediment quality assessments, the onus should be on advocates of subsampling, including authors of toxicity test protocols, to prove their case. They will rarely be able to do so. Even when subsampling is cost effective, there are often better alternatives to increase power and robustness, such as analyzing composites, using blocking factors, or testing additional factors or variables. Furthermore, the excessive subsampling commonly used in sediment quality assessments often leads to pseudoreplication or replication at inappropriate spatial or temporal scales.

Criteria Development

Overview of Environmental Quality Guidelines in Canada. U. Schneider. Environment Canada, Guidelines and Standards Division, Hull, QC.

Environmental quality guidelines (EQGs) have a long history in Canada. In the 1970s, several agencies such as Environment Canada, Health Canada, and various Provincial Ministries started to develop objectives and guidelines for the protection of different water resources such as drinking water and recreational water uses, agricultural irrigation water and livestock watering, and freshwater life. In 1987, the Canadian Council of Ministers of the Environment (CCME), a federal, provincial, and territorial council, published the Canadian Water Quality Guidelines, a compilation of nationally approved guidelines for use in Canada. These guidelines were the result of co-operative work among federal, provincial, and territorial governments. That document replaced all other previously published national water quality guidelines in Canada, and has been updated periodically with appendices of new or revised guidelines (last updated in June 1997). In the 1990s, the CCME began to address concerns regarding other media with the development of environmental quality guidelines for marine waters, sediment, soil, air, and tissue residue guidelines for the protection of wildlife consumers of aquatic biota. This broader mandate lead to the production of the "Canadian Environmental Quality Guidelines", a 1000+ page compendium of all national guidelines for environmental quality and human health in Canada. This document supersedes all previously published national environmental quality guideline values for aquatic life, sediment, soil, and tissue. This presentation will briefly describe the history of EQG development in Canada and will provide an overview of the new CCME EQG document.

Canadian Sediment Quality Guidelines. S.L. Smith, C.L. Gaudet and L.S. Juergensen. Environment Canada, Ottawa, ON.

The maintenance, protection, and restoration of a high level of environmental quality requires the availability of practical scientific tools. Canadian sediment quality guidelines, an example of such tools, are developed with the goal of no observable adverse effects on organisms living in, or on, sediments over the long-term. These national guidelines help to evaluate the toxicological significance of sediment-associated substances on aquatic life. The Canadian Council of

Ministers of the Environment (CCME) recently published a compendium of Canadian environmental quality guidelines, including freshwater and marine sediment quality guidelines for 31 substances. This presentation will provide an overview of the current status of sediment quality guideline development in Canada, the principles and processes behind sediment quality guideline development and how these processes might be influenced by new scientific information being generated as well as various political factors, and how the guidelines are being used to help make sound decisions in the assessment of sediment quality.

Canadian Soil Quality Guidelines. L.S. Juergensen[1], C.L. Gaudet[1], S.L. Smith[1], T. Schneider[2] and D. Nadon[3]. [1]Environment Canada, Ottawa, ON; [2]Environmental Consultant, Ottawa, ON; [3]Nadec Experts and Consultants Inc., Vanier, ON.

The Canadian Council of Ministers of the Environment (CCME) recently published a compendium of Canadian environmental quality guidelines, including soil quality guidelines for 25 substances. These national soil quality guidelines are derived using current toxicological data, and are intended to be protective of ecological and human health. They provide the scientific basis for the assessment and remediation of soil, based on specific land uses (agricultural, residential/parkland, commercial and industrial) and generic exposure scenarios. The formal protocol for the derivation of environmental and human health quality guidelines, first published in 1996, continues to evolve with development of new science and scientific techniques as do the frameworks by which the guidelines are applied. This presentation will provide an update of the efforts made by the Guidelines and Standards Division towards the protection of Canada's soil resources and the many beneficial uses they support, an overview of emerging areas in national soil quality guideline development, and some of the urgent scientific questions facing us in effectively protecting soil quality.

Ecotoxicity of Aqueous Film Forming Foams (AFFF) and Guideline Development for Aqueous Film Forming Foams (AFFF). I.J.Young and H. Fanous. Department of National Defence, Ottawa, ON.

Although aqueous film forming foams (AFFF) serve a vital role in combating flammable, hydrocarbon fuel based fires, there has been increasing environmental concern about AFFF effects on the aquatic environment. Comprehensive AFFF toxic effects on vertebrate, invertebrate and plant species will be presented. Secondly, the development of new guidelines will be addressed in accordance with the Canadian Council of the Ministers of the Environment (CCME) Protocol for Water Quality Guidelines for Protection of Aquatic Life. Available future options will be discussed after comparison of the new toxicity guidelines with existing requirements of American military and CGSB (ULC) specifications.

The Development of a Water Quality Index for Agricultural Streams. A-M. Anderson[1], K.A. Saffran[1], C.R. Wright[2], R.D. Neilson[3], N.D. MacAlpine[3] and S.E. Cooke[3]. [1]Alberta Environment; [2]Wright Consulting, Edmonton, AB; [3]Alberta Agriculture, Food, Rural Development, Edmonton, AB.

Alberta Agriculture, Food and Rural Development (AAFRD) has adopted water quality as a departmental performance measure and requires an index to report on the quality of agricultural streams. A prototype of the National Water Quality Index (NWQI), originally derived from British Columbia's water quality index, was the starting point for developing the Alberta Agricultural Water Quality Index (AAWQI). The formula incorporates three factors representing key aspects of water quality: the number of variables not meeting objectives (scope); the number of times objectives are not met (frequency); and the amount by which objectives are not met (amplitude). Water quality data collected from agricultural streams through the Canada Alberta Environmentally Sustainable Agriculture (CAESA) and AESA agreements were used to test various objective sets and index formulations. These data represent key variables that are directly influenced by agricultural activities. As such, they are negatively correlated to a measure of agricultural intensity, independently derived from fertiliser expenses, pesticide expenses and animal density for each watershed. It was expected that the index values would be similarly correlated to agricultural intensity. Index values generated by the NWQI were best correlated to agricultural intensity when baseline data rather than official guidelines were used as objectives. However, the index did not respond consistently to changing concentrations of water quality variables. Modifications to the calculation of frequency and amplitude eliminated this disadvantage. The resulting AAWQI yields values that are strongly correlated to agricultural intensity and are sensitive to temporal and spatial differences in water quality.

Y2K Bugs and Other Assesment Criteria for Disposal at Sea. L.M. Porebski[1], J.M. Osborne[1] and K.G. Doe[2]. [1]Environment Canada, Marine Environment Division, Ottawa, ON; [2]Environment Canada, Toxicology Laboratory, Moncton, NB.

It has been over ten years since Environment Canada realized the need to develop assessment criteria for ocean disposal permit applications, which went beyond the strict use of bulk chemical numbers. Significant progress has been made towards using a combination of effects based chemical criteria (TELs) and biological tests (toxicity and bioaccumulation tests). A concerted effort has gone into the development of guidelines, protocols and reference methods, to enable fair and consistent decision-making on disposal at sea materials. Procedures for development of the tools have been standardized. QA/QC and field validation studies have been undertaken to aid with interpretation. As EC approaches the deadline for incorporating the new assessment tools into a formal regulatory context for ocean disposal, however, several challenges remain. This presenation looks at our research, including the latest analyisis of two comprehensive pollution gradient studies, our conclusions from it, our current status and the decisions we must now take to move from a research base to a regulatory base in the new millenium.

Making Inferences from a Suite of Biological Tests. B.A. Zajdlik[1], L.M. Porebski[2], K.G. Doe[3] and J.M. Osborne[2]. [1]Zajdlik & Associates, Rockwood, ON; [2]Environment Canada, Marine Environment Division, Ottawa, ON; [3]Environment Canada, Toxicology Laboratory, Moncton, NB.

The evaluation of sediment quality using a triad approach is well established in Canada. A triad study was conducted under a known gradient of polycyclic aromatic hydrocarbons in Sydney Harbour, Nova Scotia during July of 1997, in order to confirm the interpretation criteria being promulgated by Environment Canada for a suite of marine sediment biological tests. A variety of statistical tools were used to make inferences from the sometimes complementary, contradictory or uninformative triad components. While making these inferences it became apparent that the carefully chosen reference station did not behave as a reference station with respect to the *in situ* benthic community structure. As the choice of reference station is critical in determining the relative performance of sediment evaluation tools, and also in determining effluent effects, the definition of a reference condition was revisited. The relative strengths and weaknesses of the various statistical tools in making inferences from a suite of biological tests when the unexpected arises, is discussed.

Fate, Behaviour, and Toxicity of Dioxins and Furans in the Canadian Environment: Risks to Aquatic Life and Wildlife. S.L. Roe[1], B. Koenig[2], H. Singleton[3], K. Keenleyside[1], D.D. MacDonald[4], D.E. Andersen[1], S.L. Walker[5], L.S. Juergensen[1], S.L. Smith[1] and R.S. Teed[6]. [1]Environment Canada, Guidelines and Standards Division, Hull, QC; [2]Trent University, Peterborough, ON; [3]Water Management Branch, British Columbia Ministry of the Environment, Victoria, BC; [4]MacDonald Environmental Sciences Ltd., Ladysmith, BC; [5]Environment Canada, Environmental Effects Monitoring, Hull, QC; [6]The Cadmus Group Inc., Ottawa, ON.

Canadian Environmental Quality Guidelines (EQGs) for polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) provide benchmark levels for water, sediment, and tissue above which aquatic life and/or wildlife predators of aquatic life may be at risk of adverse effects. To develop the EQGs, a detailed assessment of chemical and physical properties, fate, behaviour, Canadian environmental levels, sources, toxicity, and guidelines from other jurisdictions was completed. This information was used in accordance with protocols established by the Canadian Council of Ministers of the Environment (CCME) for deriving water and sediment quality guidelines for the protection of aquatic life and tissue residue guidelines for the protection of wildlife consumers of aquatic life. Guidelines for water, sediment, and tissue were developed concurrently for the first time, to ensure consistency among media. The theory of equilibrium partitioning was applied to cross-check guideline values across media. Moreover, to accommodate the complex nature of PCDDs and PCDFs, specifically differences in concentration and toxicity among individual congeners and between species, the EQGs were developed using those dioxin toxic equivalency factors (TEFs) for fish, mammalian, and avian species published by the World Health Organization (WHO) in 1998. The EQGs for PCDDs and PCDFs are currently undergoing peer review; details of the approach and final guideline values will be presented.

A Canada-Wide GIS Analysis of Methylmercury in Fish - Exploring Relative Risks to Wildlife and Human Health. P-Y. Caux[1], B. Miskimmin[2], H. Morrison[3], J. Parks[4], R. Post[5], K. Stone[6] and S.L. Roe[1]. [1]Environment Canada, Guidelines and Standards Division, Hull, QC; [2]Limnos Freshwater Consultants, Vernon, BC; [3]Aqualink, Toronto, ON; [4]DAMSA, Nolalu, ON; [5]Environment Canada, Knowledge Integration Division, Hull, QC; [6]SEIS, Ottawa, ON.

Much of the basis for recent action on mercury in North America stems from the extensive documentation of elevated levels of its most toxicologically-relevant form, methylmercury (MeHg), in fish, and the subsequent risks these concentrations pose to human and environmental health. Irrespective of the anthropogenic or natural sources, the primary pathway of MeHg exposure in humans and sensitive wildlife is through ingestion of contaminated fish and other aquatic life. A national master database of mercury concentrations in fish is has been developed wherein 200,000+ data points have been incorporated from most regions of Canada. A GIS-based analysis depicting spatial and temporal variation in fish tissue MeHg levels for fish species and size classes relevant to heron, osprey, kingfisher, and mink has been recently initiated; the national tissue residue guidelines serve as reference values to help and communicate the relative risks to wildlife and human health. In addition, this analysis will aid in addressing the relative contributions from human activities vs natural processes, the benefits to Canadian ecosystems from controls to date and the extent to which the current science can predict the benefits from further control actions.

Communicating Water Quality Information with an Index: the Alberta Experience. K.A. Saffran[1], A-M. Anderson[1] and C.R. Wright[2]. [1]Alberta Environment; [2]Wright Consulting, Edmonton, AB.

Alberta has for several years used a water quality index as a performance measure to report the quality of its major rivers to the general public. This simple index is derived from the percentage of concentrations that comply with Alberta's Ambient Surface Water Quality Interim Guidelines. Water quality indices have recently received much attention in Canada. The Canadian Water Quality Index (CWQI) and the Alberta Agricultural Water Quality Index (AAWQI) have recently been developed based on a formulation originally developed in British Columbia. These indices incorporate three factors representing key aspects of water quality: the number of variables not meeting objectives (scope); the number of times objectives are not met (frequency); and the amount by which objectives are not met (amplitude). Alberta Environment intends to adopt a similar index formulation. The range of variables used in the new index is broader than that used in the old AWQI. The choice of objectives includes Canadian Water Quality Guidelines, background levels and detection limits, as well as Alberta guidelines. Long-term provincial and basin specific data are used to compare results derived from the old and the new Alberta Water Quality Index (AWQI) and to illustrate the potential of the latter for depicting temporal and spatial. The advantages and disadvantages of using this index to communicate water quality information are assessed. If it is applied appropriately and if the resulting values are set in the proper context, the AWQI could become a powerful tool for describing water quality in Alberta.

Assessment of Chemicals and Products

Toxicological Evaluation of Tire Crumb for use in Public Playgrounds. D.A. Birkholz[1], K. Belton[2] and T. Guidotti[3]. [1]Enviro-Test Laboratories, Edmonton, AB; [2]Alberta Centre for Injury Control and Research, University of Alberta, Edmonton, AB; [3]Department of Environmental and Occupational Health, School of Public Health and Health Services, The George Washington University, Washington, DC.

Approximately two million tires are generated in Alberta annually and in order to recycle 100% of spent tires, new uses will have to be found. One such proposed use is in playgrounds following crushing of the tires to produce a crumb. The advantage of using tire crumb in playgrounds, as opposed to sand and/or asphalt, is that it reduces injuries to children using playground facilities. The use of tire crumb has raised concern with respect to exposure of children to unknown chemicals associated with tire crumb as well as the environmental impact associated with offsite migration of such chemicals. A comprehensive study was designed to evaluate and address potential human health and environmental concerns following the use of tire crumb in playgrounds. Human health concerns were addressed using conventional risk analyses supplemented by toxicity testing of solvent extracted tire crumb. A battery of tests was applied which measure DNA- and/or chromosome damage. Environmental concerns were addressed by testing water leachates obtained from tire crumb samples as well as a playground. Testing was performed using a battery of aquatic bioassays representative of the aquatic ecosystem. The results of the risk analysis and toxicity testing of solvent extracted tire crumb concluded that the risk associated to children was small. Testing of the water leachates revealed toxicity to all aquatic organisms (bacteria, invertebrates, fish and green algae), however, this was a transient, or short-term response. The chemicals associated with such a response were readily biodegraded, under conservative assumptions. Based upon this study it was concluded that the use of tire crumb in playgrounds results in minimal risk to children and the receiving environment and that the benefit associated with reduced injuries should encourage such usage.

Environmental Relevance of Methods For Assessing The Aquatic Toxicity Of Insoluble or Sparingly-Soluble Materials. J.E. Schroeder[1] and R.N. Hull[2]. [1]Beak International Inc., Brampton, ON; [2]Cantox Environmental Inc., Mississauga, ON.

Aquatic toxicity testing is often required when chemicals and polymers are undergoing New Substance Notification. Many of these new chemicals and polymers are insoluble or sparingly-soluble materials and, therefore, they must be solubilized prior to conducting the toxicity test. Solubilization methods, such as addition of a chemical carrier, dispersion by ultrasonification or extraction through the preparation of a water-accommodated or water-soluble fraction, influence the expression of a material's toxicity. The appropriateness of the chemical preparation method depends on several factors: the purpose of the toxicity test; compatibility of the method to the physical/chemical characteristics of the material; the expected use of the material and thus the typical environmental release scenarios as a pure product or a formulation. In spite of this, limited guidance is currently available from Canadian, U.S. or international regulatory agencies for conducting toxicity tests using insoluble or sparingly soluble materials, even though results of toxicity tests conducted on such materials are used to assess their environmental hazard. In fact, the selection of a solubilization method may have implications to both the government and the proponent. Key issues to be addressed in the selection of an appropriate preparation method are presented for consideration and discussion.

CEPA Good Laboratory Practice Compliance Monitoring Program: What's New? A. Steenkamer. Environment Canada, Environmental Technology Centre, Ottawa, ON.

Good Laboratory Practice (GLP) is an internationally recognized quality system that applies primarily to private sector facilities conducting non-clinical health and environmental safety studies for regulatory purposes. The Organisation for Economic Co-operation and Development (OECD) has developed the Principles of GLP which are intended to ensure that regulatory assessments are based on data of verifiable quality. For the last 5 years, Environment Canada has been developing a compliance monitoring program in support of the New Substances Notification Regulations based on OECD GLP Principles and related guidance documents. This presentation will update the development of Environment Canada's Canadian Environmental Protection Act (CEPA) GLP Compliance Monitoring Program and identify the latest revisions to the OECD Principles.

Effect of Microtox® Reagent Reconstitution Age on the Variability of Analytical Results from the Microtox® Assay. I.D. Gaudet and R.N. Coleman. Alberta Research Council, Vegreville, AB.

Abstract

The effect of reconstitution age and lot to lot variation of Microtox® reagent on the sensitivity and reproducibility of the assay was evaluated using four different samples (zinc sulphate, copper sulphate, phenol, sodium dodecyl sulphate) analyzed in triplicate with three reagent lots at seven different reconstitution times (0.5-24 h) using the same vial of reagent. All possibilities of an increase, decrease, and no change in toxicity with reagent age were observed. There was also lot to lot variation in toxicity results for some of the compounds. Recommendations made were: perform quality control testing on each reagent lot using standard reference toxicant(s); record the time of reconstitution of reagent as well as the start time of the assay; and reagent should not be used for testing after 4 h from time of reconstitution or with the use of an appropriate control toxicant run in parallel. These points be adopted within the standard operating procedure of the Western Canada Microtox® Users Committee (WCMUC) as part of their ongoing inter-laboratory quality control program.

Introduction

The Microtox® basic test assay has been used to determine the toxicity of such diverse samples as oil sands tailings water (MacKinnon and Retallack, 1982; Baddaloo, 1986), landfill leachates (Cameron *et al.*, 1982; Baker, 1985), pulp and paper effluent (Blaise *et al.*, 1987), drilling fluids (Strosher, 1984) and several industrial effluents (Bulich *et al.*, 1981; Vasseur *et al.*, 1984; Coleman and Qureshi, 1985). It has been generally accepted that there is no universal bioassay which can be used in every situation for toxicity testing and that a battery of screening tests should be used. Due to the ease of use, low cost, sensitivity, and reproducibility, the Microtox® test system has often been used as a pre-screening tool.

Several refinements to the Microtox® basic test assay have been made since its inception. One of the first was the use of colour correction to determine the toxicity of highly coloured samples (Beckman, 1982). In a study on the refinement of the Microtox® assay, Qureshi *et al.* (1984) made several recommendations for the assay. First, the sample pH should be considered part of the assay since adjustments cause changes within the sample. Secondly, the 15 min EC_{50} should be accepted as the standard endpoint for assaying samples, particularly those containing metallic ions. These two recommendations have found general acceptance. The third major recommendation, that freshly reconstituted reagent be used for testing, does not seem to have been generally accepted. This may be due to the expense of the reagent, time constraints within a testing laboratory, or other unknown factors. A recent publication lists the following bacterial factors as causes of variability of the toxicity test results: age of freeze-dried bacteria, bacterial storage and conservation, reconstitution procedure, age of the reconstituted reagent, and temperature equilibration time (Ribo, 1997). However, Ribo did not present any results which would address these issues.

The objectives of this study were to: investigate the effect of reconstitution age of Microtox® test reagent on the results obtained with the assay; and investigate the effect of lot to lot variation of Microtox® test reagent, and provide a possible recommendation as to the maximum time after reconstitution for use of the Microtox® test reagent. The scope of the work plan for each of the components was as follows. This study evaluated four different samples at seven different reconstitution times between 0.5-24 h (0.5, 0.75, 1.0, 2.0, 4.0, 8.0, 24.0) using the same vial of reagent. The samples consisted of two metallic salts, copper sulphate and zinc sulphate and two organics, phenol and sodium dodecyl sulphate (SDS). Each sample was analyzed in triplicate. This series was repeated with three different reagent lots, AVC009-2, AVC009-3, and AVC010-3. Statistical analysis of results focused on 15 min EC_{50} data only since this is the time point most commonly used for reporting of Microtox® testing results.

Materials and Methods

Microtox® Analysis: The analytical procedure for using the Model 500 analyzer for Microtox® testing has been described previously (Gaudet, 1994). The assays were performed using the standard 82% assay (AEC variation) (Gaudet, 1994). Timing for the assays was determined as follows. Reagent was reconstituted, the clock was started and reagent was allowed to sit in the reagent well for 15 min. Reagent was then diluted into the testing wells and allowed to equilibrate for an additional 15 min. Initial light readings were taken and, at 30 min from the time of reconstitution, the toxicant was added to the testing wells. Time was determined as the total elapsed time from reconstitution to the addition of the toxicant, allowing for temperature equilibration. To run additional assays from the same reagent vial, the reagent was again diluted into testing wells and allowed to equilibrate to run analysis at the additional time points. Reagent was added to diluent 15 min prior to running an assay to allow for temperature equilibration.

Statistical Analysis: For the statistical analysis, two questions were asked. Firstly, "is there a difference between the toxicity values at the various time points?" In order determine this statistical comparison, a standard time of 1 h was chosen and all other results were compared to this standard time. The second question was "is there a difference between the reagent lots at each time point?"

Differences in EC_{50} values at various time points against the time point "1 h." The statistical model for each reagent was the One-way Analysis of Variances. The general form of the model is given by

$[EC_{50}]_{ijk} = \mu + \beta_{ij}*[Time(h)]_{ijk} + \varepsilon_{ijk}$, where μ = overall mean,
β_{ij}, j = 0.5, 0.75, 1, 2, 4, 8, 24 = main effect of time for each
i = ACV009-2, ACV009-3, ACV010-3, $\varepsilon_{ijk} \sim$ iid $N(0,\sigma^2)$ with k = 1, 2, 3.

For each reagent i, the mean values of EC_{50} of various time points were compared against the mean EC_{50} of normal time 1 h" using Dunnett's comparison method in GLM procedure in SAS/STAT software (SAS Institute Inc. 1990a, 1990b, 1994).

Differences of EC_{50} values between reagent lots at each time point. The objective of this statistical analysis was to examine whether EC_{50} values of different reagents are significantly different at each time point. The statistical model for this analysis was the One-way Analysis of Covariance. The general form of the model is given by

$[EC_{50}]_{ijk} = \mu + \alpha_i + \beta_j*[Time(h)]_{ijk} + (\alpha\beta)_{ij} + \varepsilon_{ijk}$, where μ = overall mean,
α_i, i = ACV009-2, ACV009-3, ACV010-3 = main effect of Reagent,
β_j, j = 0.5, 0.75, 1, 2, 4, 8, 24 = main effect of Time,
$(\alpha\beta)_{ij}$ = interaction between Reagent and Time,
$\varepsilon_{ijk} \sim$ iid $N(0,\sigma^2)$ with k = 1, 2, 3.

After the model was fitted using GLM procedure in SAS/STAT software from SAS institute (SAS

Institute Inc. 1990a, 1990b, 1994), the adjusted means of EC_{50} for each reagent within each time point were compared against each other using Tukey's method.

Results

Zinc: The overall mean EC_{50} 15 min for Zn sulphate was 1.291 mg/L Zn^{+2} for all reconstitution times (Fig. 1). However, a sharp increase in toxicity was observed at 24 h and these assays had an overall EC_{50} of 0.518 mg/L Zn^{+2}. When the 24 h data were removed, the mean EC_{50} was 1.420 mg/L Zn^{+2}. AZUR certificates of performance list zinc sulphate EC_{50} 15 min 0.6 to 2.2 mg/L Zn^{+2}. All assays, except for the majority of those performed at 24 h, fall into these general specifications. In addition, most assays results were similar to the AZUR quality assurance results which list the zinc sulphate EC_{50} 15 min (mg/L Zn^{+2}) at 1.3 for lot ACV009-2, 1.0 for lot ACV009-3, and 1.2 for lot ACV010-3. EC_{50} results for this study ranged from a low of 0.835 to a high of 2.123 (Table 1). Statistical analysis of EC_{50} results compared to the chosen standard time of 1 h found that for all lots, the 24 h data was significantly different (Table 2). Therefore, the rejection of 24 h data was statistically reinforced. In addition, for lot ACV009-2 the EC_{50} results for 0.5, 0.75 and 8 h were significantly different from the 1 h results (Table 2). However, when adjusted means of EC_{50} results were compared, there was no difference between the 3 reagent lots at all time points tested (Table 3).

Table 1. Zinc sulphate EC_{50} 15 min data summary (mg/L Zn^{+2}).

		Time (h)							
Reagent	Replicate	0.5	0.75	1	2	4	8	24	Mean
ACV009-2	1	1.243	1.791	1.452	2.123	1.875	1.204	0.692	1.483
	2	1.151	1.106	1.502	1.633	1.377	1.000	0.498	1.181
	3	1.108	1.382	1.540	1.711	1.621	1.182	0.640	1.312
	Mean	1.167	1.426	1.498	1.822	1.624	1.129	0.610	1.325
ACV009-3	1	1.367	1.101	1.584	1.970	1.497	1.027	0.545	1.299
	2	0.838	1.015	1.709	1.229	1.278	0.920	0.548	1.077
	3	1.099	0.835	1.887	1.653	1.420	0.896	0.469	1.180
	Mean	1.101	0.984	1.727	1.617	1.398	0.948	0.521	1.185
ACV010-3	1	1.123	1.761	1.893	1.407	1.592	1.607	0.383	1.395
	2	1.706	1.494	1.489	1.333	1.883	1.456	0.519	1.411
	3	1.625	1.173	1.534	1.754	1.309	1.201	0.368	1.281
	Mean	1.485	1.476	1.639	1.498	1.595	1.421	0.423	1.362

Table 2. Zinc sulphate differences among mean EC_{50} values (mg/L Zn^{+2}).

		Time (h)						
Reagent		0.5	0.75	1	2	4	8	24
ACV009-2	Arithmetic Mean	1.17	1.43	1.50	1.82	1.62	1.13	**0.61***
	Standard Error	0.12	0.12	0.12	0.12	0.12	0.12	0.12
ACV009-3	Arithmetic Mean	**1.10***	**0.98***	1.73	1.62	1.40	**0.95***	**0.52***
	Standard Error	0.11	0.11	0.11	0.11	0.11	0.11	0.11
ACV010-3	Arithmetic Mean	1.48	1.48	1.64	1.50	1.59	1.42	**0.42***
	Standard Error	0.14	0.14	0.14	0.14	0.14	0.14	0.14

Within each reagent, symbol "*" indicates that the mean is significantly different from the mean for 1 h duration at 95% confidence level.

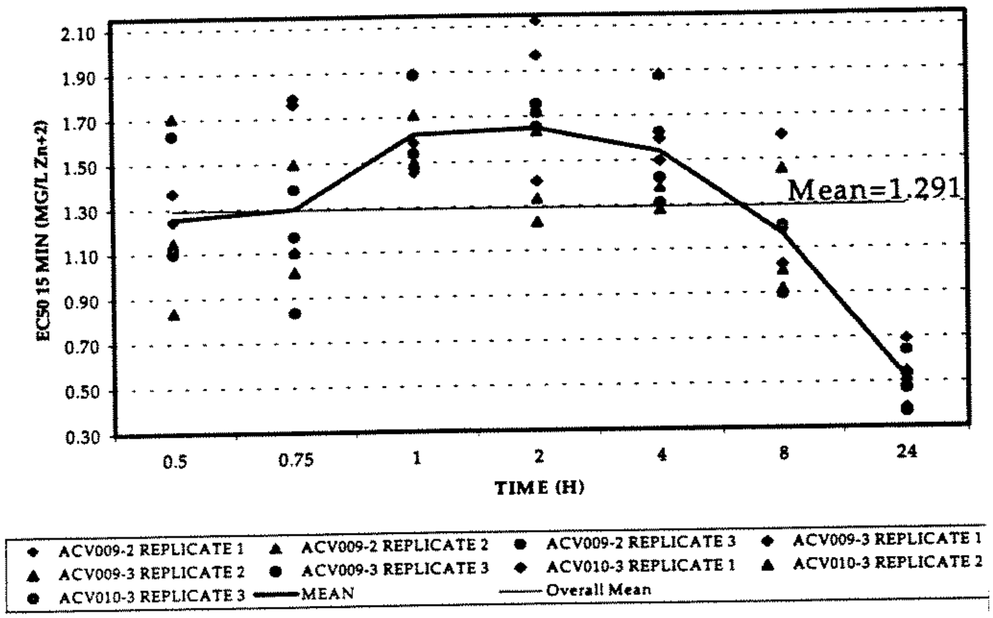


Fig. 1. Zinc sulphate EC_{50} 15 minute data

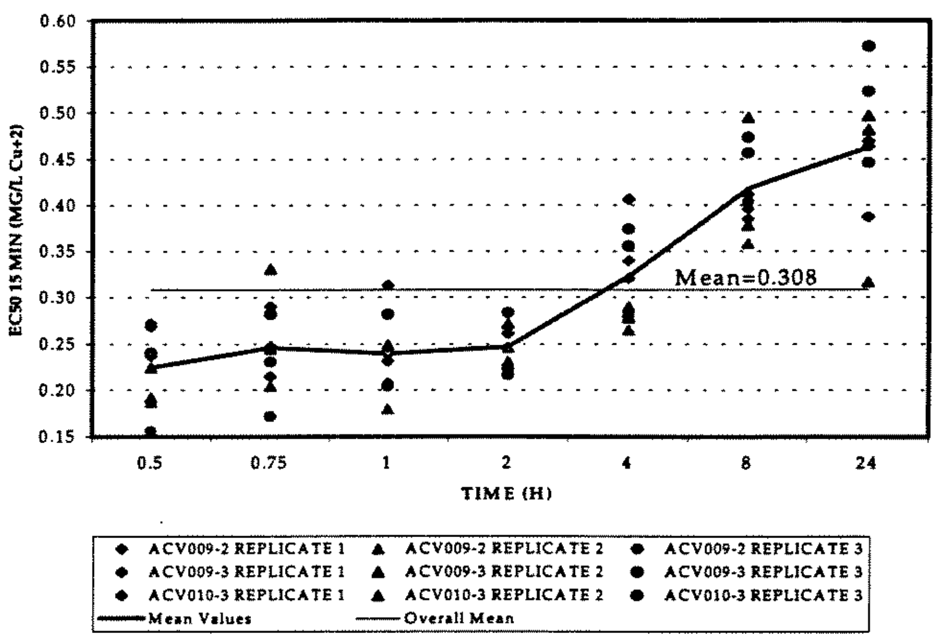


Fig. 2. Copper sulphate EC_{50} 15 minute data

Table 3. Zinc sulphate differences among means, reagent lots EC_{50} values (mg/L Zn^{+2}).

Time (h)		Reagent ACV009-2	ACV009-3	ACV010-3	Multiple
0.50	Adjusted Mean	1.53	1.38	1.61	aaa
	Standard Error	0.08	0.08	0.08	
0.75	Adjusted Mean	1.52	1.37	1.60	aaa
	Standard Error	0.07	0.07	0.07	
1.00	Adjusted Mean	1.51	1.36	1.59	aaa
	Standard Error	0.07	0.07	0.07	
2.00	Adjusted Mean	1.47	1.32	1.54	aaa
	Standard Error	0.07	0.07	0.07	
4.00	Adjusted Mean	1.39	1.25	1.45	aaa
	Standard Error	0.06	0.06	0.06	
8.00	Adjusted Mean	1.24	1.10	1.26	aaa
	Standard Error	0.06	0.06	0.06	
24.00	Adjusted Mean	0.63	0.52	0.50	aaa
	Standard Error	0.16	0.16	0.16	

Within each time point, different letters indicate that the adjusted means are significantly different from each other at the 95% confidence level, i.e. no reagent means at a given time are different when the three letters are the same.

Copper: Copper sulphate had an overall mean EC_{50} at 15 min of 0.308 mg/L Cu^{+2} for all reconstitution times (Fig. 2). However, a sharp decrease in toxicity was observed at 24 h (overall EC_{50} of 0.461 mg/L Cu^{+2}). When the 24 h data were removed, the mean EC_{50} was 0.282 mg/L Cu^{+2}. The decrease in toxicity was still observed at 8 h (overall EC_{50} of 0.417 mg/L Cu^{+2}). When the 24 and 8 h data were removed, the mean EC_{50} was 0.256 mg/L Cu^{+2} ranging from a low of 0.156 to a high of 0.406 (Table 4). Statistical analysis of EC_{50} results compared to the chosen standard time of 1 h found that, for all lots, the 8 and 24 h data were significantly different (Table 5). Thus, the rejection of these time points was statistically reinforced. In addition, the comparisons of adjusted means of EC_{50} results found that there was a difference between the 3 lots at the 4h and 8 h time points (Table 6).

Phenol: Phenol had an overall mean EC_{50} 15 min of 21.366 and ranged from a low of 16.601 to a high of 27.061 (Table 7). The graphical plot of these results (Fig. 3) shows no particular trend in toxicity values, however, there does appear to be lot to lot variation with results from lot ACV009-2 being consistently high and results from lot ACV010-3 being consistently low. Statistical analysis of EC_{50} results compared to the chosen standard time of 1-h found that significantly different results were observed at 2 and 24 h for reagent lot AVC009-2 and at 0.75 and 2 h for reagent lot ACV010-3 (Table 8). In addition, adjusted means of EC_{50} results comparison finds that there was a difference between the 3 lots at all time points except for 24 h (Table 9). This reaffirms the observation of high/low distribution with particular reagent lots seen in Fig. 3.

Table 4. Copper sulphate EC_{50} 15 min data summary (mg/L Cu^{+2}).

		Time (h)							
Reagent Lot	Replicate	0.5	0.75	1	2	4	8	24	Mean
ACV009-2	1	0.272	0.247	0.208	0.268	0.340	0.396	0.387	0.303
	2	0.187	0.204	0.180	0.231	0.265	0.358	0.316	0.249
	3	0.156	0.172	0.204	0.222	0.279	0.404	0.523	0.280
	Mean	0.205	0.208	0.197	0.240	0.295	0.386	0.409	0.277
ACV009-3	1	0.237	0.214	0.231	0.222	0.406	0.411	0.463	0.312
	2	0.192	0.244	0.249	0.271	0.278	0.495	0.496	0.318
	3	0.240	0.282	0.282	0.283	0.356	0.456	0.572	0.353
	Mean	0.223	0.247	0.254	0.259	0.347	0.454	0.510	0.328
ACV010-3	1	0.268	0.290	0.313	0.261	0.320	0.385	0.469	0.329
	2	0.224	0.331	0.245	0.246	0.290	0.378	0.481	0.314
	3	0.240	0.230	0.240	0.216	0.374	0.473	0.446	0.317
	Mean	0.244	0.284	0.266	0.241	0.328	0.412	0.465	0.320
	Overall Mean	0.224	0.246	0.239	0.247	0.323	0.417	0.461	0.308

Table 5. Copper sulphate differences among mean EC_{50} values (mg/L Cu^{+2}).

		Time (h)						
Reagent		0.5	0.75	1	2	4	8	24
ACV009-2	Arithmetic Mean	0.21	0.21	0.20	0.24	0.29	**0.38***	**0.41***
	Standard Error	0.03	0.03	0.03	0.03	0.03	0.03	0.03
ACV009-3	Arithmetic Mean	0.22	0.25	0.25	0.26	0.35	**0.45***	**0.51***
	Standard Error	0.02	0.02	0.02	0.02	0.02	0.02	0.02
ACV010-3	Arithmetic Mean	0.24	0.28	0.27	0.24	0.33	**0.41***	**0.47***
	Standard Error	0.02	0.02	0.02	0.02	0.02	0.02	0.02

Within each reagent, symbol "*" indicates that the mean is significantly different from the mean for 1 h duration at 95% confidence level.

Sodium dodecyl sulphate: SDS had an overall mean EC_{50} at 15 min of 0.893 mg/L for all reconstitution times (Fig. 4). However, a sharp decrease in toxicity was observed at 24 h (overall EC_{50} of 1.708 mg/L). When the 24 h data were removed, the mean EC_{50} was 0.757 mg/L ranging from a low of 0.503 to a high of 1.003 (Table 10). Statistical analysis of EC_{50} results compared to the chosen standard time of 1 h found that for all lots, the EC_{50} at 24 was significantly different from that at 1 h (Table 11). Therefore, the rejection of 24 h data as presented in Fig. 9 is statistically reinforced. In addition, adjusted means of EC_{50} comparison, finds that there was a difference between the 3 lots at 8 and 24 h (Table 12).

Discussion

The first and most obvious conclusion to be drawn from this testing was that there were differences in toxicity over time for the compounds tested. Zn exhibited a sharp increase in toxicity at 24 h, however EC_{50} results were relatively stable prior to that time. EC_{50} ranged from a low of 0.835 to a high of 2.123 with the mean value excluding 24 h data being 1.420. SDS also exhibited an increase in toxicity at 24 h. Results prior to this time point were fairly stable as well. EC_{50} ranged from a low of 0.503 to a high of 1.003 with the mean value

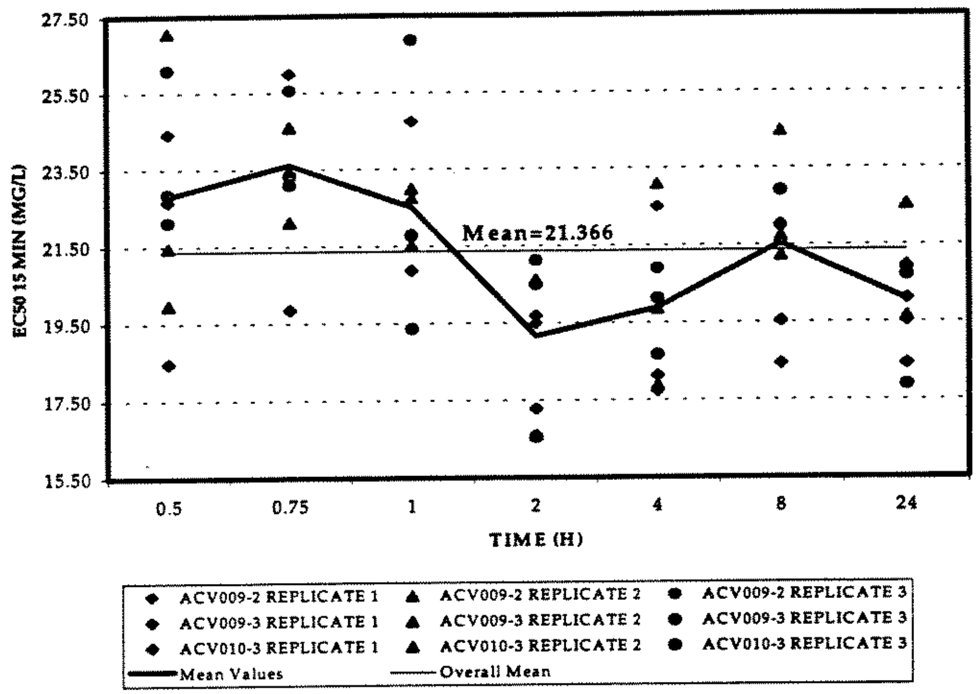


Fig. 3. Phenol EC_{50} 15 minute data

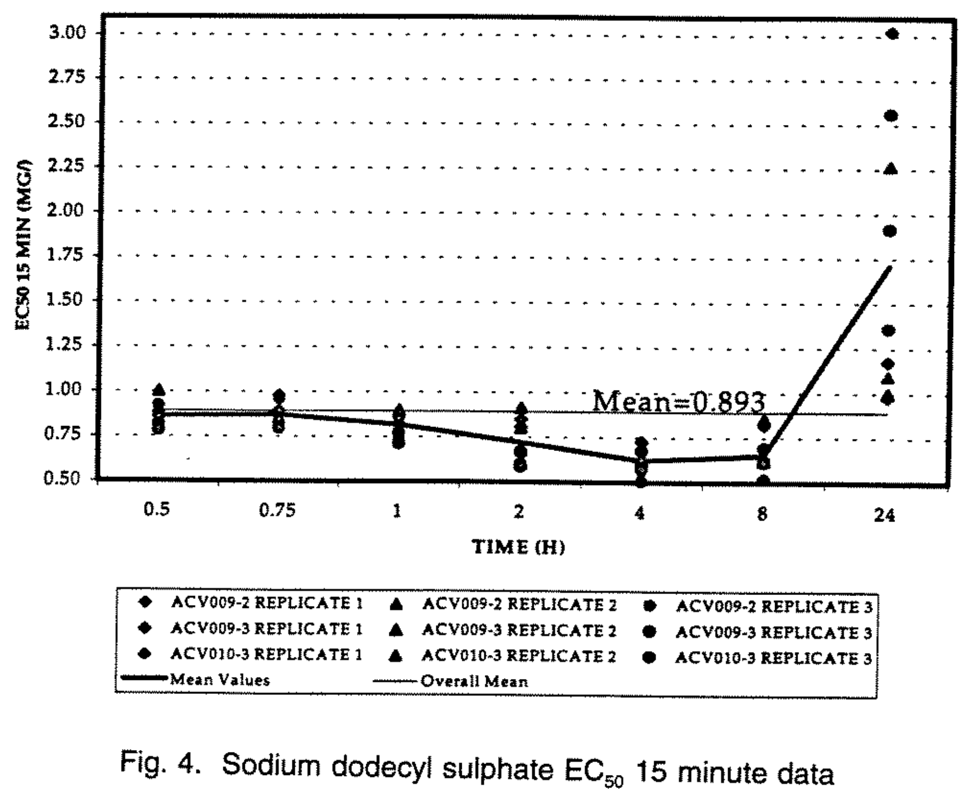


Fig. 4. Sodium dodecyl sulphate EC_{50} 15 minute data

Table 6. Copper sulphate differences among means, reagent lots EC_{50} values (mg/L Cu^{+2}).

		Reagent			
Time		ACV009-2	ACV009-3	ACV010-3	Multiple
0.50	Adjusted Mean	0.23	0.26	0.27	aaa
	Standard Error	0.02	0.02	0.02	
0.75	Adjusted Mean	0.23	0.27	0.27	aaa
	Standard Error	0.02	0.02	0.02	
1.00	Adjusted Mean	0.23	0.27	0.28	aaa
	Standard Error	0.01	0.01	0.01	
2.00	Adjusted Mean	0.24	0.28	0.29	aaa
	Standard Error	0.01	0.01	0.01	
4.00	Adjusted Mean	0.26	0.31	0.30	**aba**
	Standard Error	0.01	0.01	0.01	
8.00	Adjusted Mean	0.30	0.35	0.34	**aba**
	Standard Error	0.01	0.01	0.01	
24.00	Adjusted Mean	0.44	0.55	0.49	aaa
	Standard Error	0.03	0.03	0.03	

Within each time point, different letters indicate that the adjusted means are significantly different from each other at the 95% confidence level, i.e. no reagent means at a given time are different when the three letters are the same.

Table 7. Phenol EC_{50} 15 min data summary (mg/L).

		Time (h)							
Reagent	Replicate	0.5	0.75	1	2	4	8	24	Mean
ACV009-2	1	24.417	25.989	24.730	19.489	22.494	22.029	19.529	22.668
	2	27.061	24.596	22.753	20.580	23.085	24.457	20.984	23.359
	3	26.076	25.556	26.862	21.124	20.900	21.977	20.895	23.341
	Mean	25.851	25.380	24.782	20.398	22.160	22.821	20.469	23.123
ACV009-3	1	22.661	19.846	21.801	19.675	18.118	19.522	18.419	20.006
	2	19.968	22.130	22.991	20.614	19.858	21.736	22.539	21.405
	3	22.119	23.098	21.766	20.487	20.119	22.906	20.703	21.600
	Mean	21.583	21.691	22.186	20.259	19.365	21.388	20.554	21.004
ACV010-3	1	18.459	23.385	20.865	17.271	17.729	18.423	20.097	19.461
	2	21.464	24.600	21.526	16.601	17.880	21.228	19.651	20.421
	3	22.851	23.313	19.353	16.544	18.666	21.594	17.878	20.028
	Mean	20.925	23.766	20.581	16.805	18.092	20.415	19.209	19.970
	Overall Mean	22.786	23.613	22.516	19.154	19.872	21.541	20.077	21.366

excluding 24 h data being 0.757. Cu, on the other hand, exhibited a decrease in toxicity starting at 4 h which continued at the 8 and 24 h testing points. Prior to this, however, toxicity results were very stable. EC_{50} ranged from a low of 0.156 to a high of 0.406 with the mean value excluding 8 and 24 h data being 0.256. Phenol produced no toxicity trend over time, however, throughout the 24 h period, the results were erratic. EC_{50} ranged from a low of 16.601 to a high of 27.061 with the mean value being 21.366. Thus, based on the results of

Table 8. Phenol differences among mean EC_{50} values (mg/L).

Reagent		Time (h) 0.5	0.75	1	2	4	8	24
ACV009-2	Arithmetic Mean	25.85	25.38	24.78	**20.40***	22.16	22.82	**20.47***
	Standard Error	0.73	0.73	0.73	0.73	0.73	0.73	0.73
ACV009-3	Arithmetic Mean	21.58	21.69	22.19	20.26	19.37	21.39	20.55
	Standard Error	0.82	0.82	0.82	0.82	0.82	0.82	0.82
ACV010-3	Arithmetic Mean	20.92	**23.77***	20.58	**16.81***	18.09	20.42	19.21
	Standard Error	0.74	0.74	0.74	0.74	0.74	0.74	0.74

Within each reagent, symbol "*" indicates that the mean is significantly different from the mean for 1 h duration at 95% confidence level.

Table 9. Phenol differences among means, reagent lots EC_{50} values (mg/L).

Time		Reagent ACV009-2	ACV009-3	ACV010-3	Multiple
0.50	Adjusted mean	23.99	21.17	20.27	**abb**
	Standard error	0.53	0.53	0.53	
0.75	Adjusted mean	23.94	21.16	20.26	**abb**
	Standard error	0.52	0.52	0.52	
1.00	Adjusted mean	23.90	21.16	20.24	**abb**
	Standard error	0.52	0.52	0.52	
2.00	Adjusted mean	23.74	21.12	20.19	**abb**
	Standard error	0.49	0.49	0.49	
4.00	Adjusted mean	23.41	21.06	20.07	**abb**
	Standard error	0.45	0.45	0.45	
8.00	Adjusted mean	22.75	20.93	19.84	**abb**
	Standard error	0.46	0.46	0.46	
24.00	Adjusted mean	20.13	20.42	18.93	aaa
	Standard error	1.12	1.12	1.12	

Within each time point, different letters indicate that the adjusted means are significantly different from each other at the 95% confidence level (i.e., no reagent means at a given time are different when the three letters are the same).

all samples, a prediction of change in toxicity of an unknown compound or sample with time cannot be made. With the four compounds tested, all three possibilities of increase, decrease, or no change in toxicity were observed. The second conclusion was that Microtox® test reagent should definitely not be used at 24 h, and probably not at 8 h. Except for phenol, the 24 h data were rejected for all compounds tested and the 8 h data were rejected for Cu as well. Thus, data obtained from reagent which is older than 4 h should be regarded cautiously, if at all. When statistical analysis of data between lots was performed, it was found that there was variation in toxicity results between lots for Cu, phenol, and SDS. With phenol, for example, the results for lot ACV009-2 are consistently higher than those for the other two lots except at 24 h (Fig. 3). Phenol shows a definite trend for lot to lot variation with EC_{50} results

Table 10. Sodium dodecyl sulphate EC_{50} 15 min data summary (mg/L).

	Time (h)							
	0.5	0.75	1	2	4	8	24	Mean
1	0.855	0.953	0.736	0.654	0.721	0.632	0.973	0.789
2	0.853	0.821	0.845	0.603	0.534	0.621	1.095	0.767
3	0.920	0.873	0.770	0.598	0.503	0.465	1.359	0.784
Mean	0.876	0.882	0.784	0.618	0.586	0.573	1.142	0.780
1	0.882	0.979	0.862	0.805	0.593	0.523	1.174	0.831
2	1.003	0.852	0.900	0.801	0.629	0.625	1.001	0.830
3	0.818	0.826	0.770	0.668	0.674	0.691	1.913	0.909
Mean	0.901	0.886	0.844	0.758	0.632	0.613	1.363	0.857
1	0.860	0.895	0.854	0.848	0.727	0.819	3.024	1.147
2	0.806	0.851	0.899	0.915	0.636	0.860	2.268	1.034
3	0.782	0.794	0.710	0.584	0.573	0.610	2.562	0.945
Mean	0.816	0.847	0.821	0.782	0.645	0.763	2.618	1.042
Overall Mean	0.864	0.872	0.816	0.720	0.621	0.650	1.708	0.893

Table 11. Sodium dodecyl sulphate differences among mean EC_{50} values (mg/L).

		Time (h)						
Reagent		0.5	0.75	1	2	4	8	24
ACV009-2	Arithmetic mean	0.88	0.88	0.78	0.62	0.59	0.57	**1.14***
	Standard error	0.06	0.06	0.06	0.06	0.06	0.06	0.06
ACV009-3	Arithmetic mean	0.90	0.89	0.84	0.76	0.63	0.61	**1.36***
	Standard error	0.11	0.11	0.11	0.11	0.11	0.11	0.11
ACV010-3	Arithmetic mean	0.82	0.85	0.82	0.78	0.65	0.76	**2.62***
	Standard error	0.10	0.10	0.10	0.10	0.10	0.10	0.10

Within each reagent, symbol "*" indicates that the mean is significantly different from the mean for 1 h duration at 95% confidence level.

being lowest for lot ACV010-3 and highest for lot ACV009-2. Statistical analysis for lot to lot variation for phenol indicated there was variation at all time points except for 24 h (Table 9).

There are several recommendations which arise from this study. Many of these are part of general practice of most laboratories performing the Microtox® test. These recommendations are as follows. Each lot of reagent should have quality control testing performed using standard reference toxicant(s). This long term testing would then indicate lot to lot variation and might indicate if there is a problem with a new lot of reagent. Testing should be performed on each new lot of reagent and at regular intervals during the shelf life of that lot. Bench recording sheets or computer recording programs should include the time of reconstitution of reagent as well as the start time of the assay. In this manner, potential problems in interpretation of data caused by use of aged reagent can be avoided.

Table 12. Sodium dodecyl sulphate differences among means, reagent lots EC_{50} values (mg/L).

		Reagent			
Time		ACV009-2	ACV009-3	ACV010-3	Multiple
0.50	Adjusted mean	0.71	0.75	0.64	aaa
	Standard error	0.06	0.06	0.06	
0.75	Adjusted mean	0.71	0.75	0.66	aaa
	Standard error	0.06	0.06	0.06	
1.00	Adjusted mean	0.71	0.76	0.68	aaa
	Standard error	0.06	0.06	0.06	
2.00	Adjusted mean	0.73	0.78	0.75	aaa
	Standard error	0.06	0.06	0.06	
4.00	Adjusted mean	0.76	0.82	0.91	aaa
	Standard error	0.05	0.05	0.05	
8.00	Adjusted mean	0.81	0.90	1.21	**aab**
	Standard error	0.06	0.06	0.06	
24.00	Adjusted mean	1.04	1.24	2.44	**aab**
	Standard error	0.13	0.13	0.13	

Within each time point, different letters indicate that the adjusted means are significantly different from each other at the 95% confidence level, i.e. no reagent means at a given time are different when the three letters are the same.

Microtox® test reagent should generally not be used for testing after 4 h from time of reconstitution. Any assay performed after this time may yield unpredictable results due to reagent age and not toxicity of the sample being tested. Results obtained from reagent used after 4 h needs to be evaluated cautiously, with the use of an appropriate control toxicant run in parallel. It is further recommended that WCMUC adopt these points within their standard operating procedure and as part of their ongoing inter-laboratory quality control program.

Acknowledgements

This work was performed under contract to the Western Canada Microtox® Users Committee (WCMUC). Microtox® test reagent and analytical supplies were supplied by AZUR Environmental, Carlsbad, CA. Statistical analysis was performed by Hai Nguygen and Dr. Zack Florence, ARC Statistical Applications Program.

References

Baddaloo, E.G. 1986. Proceeding of Alberta Oil Sands Tailings Wastewater Treatment Technology Workshop. Held at Mildred Lake Research Station, Fort McMurray, Alberta; 1985 October 29-30. Prepared for Alberta Environment, Research Management Division. RMD Report 36/38. 129 pp.

Baker, B.L. 1985. Surface water contamination by landfill leachates. Prepared for Alberta Environment, Research Management Division by Kananaskis Centre for Environmental Research, University of Calgary. RMD Report L-90. 27 pp.

Beckman. 1982. Microtox® System Operation Manual #015-555879, Beckman Instruments, Inc., Carlsbad, CA.

Blaise, C., R. Van Coillie, N. Bermingham and G. Coulombe. 1987. Comparaison des responses toxiques de trois indicateurs biologiques (bacteries, algues, poissons) exposes a des effluents de fabriques de pates et papiers. Revue international des sciences de l'eau 3(1): 9-17.

Bulich, A.A., M.W. Greene and D.L. Isenberg. 1981. Reliability of the bacterial luminescence assay for determination of the toxicity of pure compounds and complex effluents, p. 338-347. *In* Aquatic Toxicology and Hazard Assessment: Fourth Conference, ASTM STP 737. *Edited by* R. Branson and K.L. Dickson. American Society for Testing and Materials, Philadelphia, PA.

Cameron, R.D., E.C. McDonald, M.G. Mager, S.C. Liptak and P.D. Parkinson. 1982. Toxicity of landfill leachates. Environ. Canada Rep. EPS 4-EC-82-7. 41 pp.

Coleman, R.N., and A.A. Qureshi. 1985. Microtox® and *Spirillum volutans* tests for assessing toxicity of environmental samples. Bull. Environ. Contam. Toxicol. 35: 443-451.

Gaudet, I.D.(ed.). 1994. Standard procedure for Microtox® analysis. Alberta Environmental Centre, Vegreville, AB. AECV94-G1. 63 pp.

MacKinnon, M.D., and J.T. Retallack. 1982. Preliminary characterization and detoxifications of tails pond water at the Syncrude Canada Ltd. oil sands plant, p. 185-210. *In* Land and Water Issues Related to Energy Development. *Edited by* P.J. Rand. Ann Arbour Sci.

Qureshi, A.A., R.N. Coleman and J.H. Paran. 1984. Evaluation and refinement of the Microtox® test for use in toxicity screening, p. 1-22. *In* Toxicity Screening Procedures Using Bacterial Systems. *Edited by* D. Liu and B.J. Dutka. Marcel Dekker Ind., New York, NY.

Ribo, J.M. 1997. Interlaboratory comparison studies of the luminescent bacteria toxicity bioassay. Environ. Toxicol. Water Qual. 12: 283-294.

SAS Institute Inc. 1990a. SAS system for linear models, Third Edition, Cary, NC: SAS Institute Inc., 329 pp.

SAS Institute Inc. 1990b. SAS/STAT User's Guide, Version 6, Fourth Edition, Vol. 2.

SAS Institute Inc. 1994. SAS/STAT Software: Changes and Enhancements, Release 6.10, Cary, NC: SAS Institute Inc., 121 pp.

Strosher, M.T. 1984. A comparison of biological testing methods in association with chemical analyses to evaluate toxicity of waste drilling fluids in Alberta. Vol. 1. Canadian Petroleum Association Calgary, AB.

Vasseur, P., J.F. Ferand, C. Rast and G. Larbaight. 1984. Luminescent marine bacteria in ecotoxicity screening test of complex effluents, p. 23-35. *In* Toxicity Screening Procedures Using Bacterial Systems. *Edited by* D. Liu and B.J. Dutka. M. Dekker Inc. New York, NY.

Biodegradability Evaluations for the Testing of Chemical Products in Canada. M. O'Reilly and G. Gilron. ESG International, Guelph, ON.

An important data requirement for the risk assessment process for chemical products is the evaluation of biodegradability, since persistence is one of the more crucial aspects of exposure to aquatic biota. Although there are numerous methods for evaluating biodegradability, the most widely used and accepted series of test methods in Canada are the Organization for Economic Cooperation and Development (OECD) 301 and 302 test series for Ready and Inherent Biodegradability, respectively. In this presentation, we provide an overview of the various methods of determining biodegradability, outlining: strengths and weaknesses of the test methods specifically with respect to various chemical "types", and the application of each of these methods. We will also discuss biodegradation of substances in natural systems, how test systems compare to natural systems, and the application of biodegradability test data for fate modelling. Moreover, we will discuss some critical aspects of the methods, including the validity criteria and their practicality, and the assumptions upon which the methods are based. In addition, we will provide the context in which these tests are conducted, including regulatory compliance programs (e.g., Environment/ Health Canada's New Substance Notification), and product stewardship initiatives (e.g., Environmental Choice Program, life cycle assessments).

Risk Assessment

Measurement of Subsurface Hydrocarbon Vapours at Upstream Oil and Gas Sites: Implications for Risk Assessment. T.L. Knafla[1], W. Stein[2], J. Sevigny[2] and L. Serbin[3]. [1]T.L. Knafla, Calgary, AB; [2]Komex International Ltd., Calgary, AB; [3]Enviro-Test Laboratories, Edmonton, AB.

A significant portion of subsurface hydrocarbon contamination detected at upstream petroleum substance release sites is composed of volatile hydrocarbons. These hydrocarbons must be addressed in risk assessments of upstream sites, particularly for situations where vapours could penetrate through the foundation of a building built on-site. Classically, risk assessments have evaluated this pathway by modeling the vapour concentration at the foundation surface based on a measured source concentration. An alternative is to directly measure the concentrations of subsurface vapours. Subsurface air samples were collected from four upstream oil and gas sites at depths ranging from 0.75-1.5 m below ground surface and were analysed using GC/MS techniques. Comparison of measured versus predicted vapour concentrations indicate that in general, predicted values exceeded measured values at the four sites. However, an adjustment of predicted concentrations based on partial pressures and mass fractions in the source yielded greater similarity between the values. Adjustment of modelled values using partial pressures and mass fractions may provide more realistic values, as demonstrated from comparison with measured data. Subsurface soil vapour results can be used directly in a risk assessment to estimate indoor air concentrations. Furthermore, subsurface soil vapour criteria can be developed and used to assist in identifying areas that may require remediation as a result of unacceptable health risks via the vapour migration pathway.

Importance of Different Aliphatic and Aromatic Petroleum Hydrocarbon Fractions in Human Health Risk Assessment. J.H. Sevigny[1], M.J. Tindal[1], G.L. Robins[1], W. Stein[1] and L. Serbin[2].

[1]Komex International Ltd., Calgary, AB; [2]EnviroTest Laboratories, Edmonton, AB.

Conventional risk assessments at gasoline contaminated sites predict adverse health effects from inhalation exposures to BTEX compounds, which comprise only 3-5% of the volatile hydrocarbon mass. We have measured volatile petroleum hydrocarbon vapours in shallow soils and indoor air at eight gasoline contaminated sites in western Canada and have identified the total hydrocarbon mass using GC/MS. The aliphatic hydrocarbon fraction comprised approximately 94% of the hydrocarbon mass in shallow soils. Potential human health effects from inhalation exposure were predicted for benzene and four hydrocarbon fractions (C_5-C_8 and C_9-C_{10} aliphatics and C_7-C_8 and C_9-C_{10} aromatics). Attenuation factors from the Johnson and Ettinger (1991) model were used to predict indoor air exposure point concentrations from soil vapour data. Exposure limits were taken from US EPA IRIS (benzene) and the United States Total Petroleum Hydrocarbon Criteria Working Group (aliphatic and aromatic fractions). Exposure Ratios were predicted for benzene and the four hydrocarbon fractions. This presentation will characterize volatile petroleum hydrocarbons in shallow soil and indoor air, and will examine Exposure Ratios for different hydrocarbon fractions predicted for a commercial setting.

Application of the Ontario Ministry of the Environment (OMOE) Whole Mixture Model for the Estimation of Human Health Risk from a PAH Mixture. A.G. Verbeek[1], G.A. Clyde[1], K.T. Himbeault[2] and J. Goodin[3] .[1]Conor Pacific Environmental Technologies Inc., Edmonton, AB; [2]#4-130 Robin Crescent, Saskatoon, SK; [3]2240 Speakman Drive, Mississauga, ON.

Human health risk for complex mixtures of Polycyclic Aromatic Hydrocarbons (PAH) is often estimated based on the sum of modeled estimates of risk for individual PAH compounds using what has been termed the Individual PAH Model (IPM). A more recent approach to the estimation of risk of a PAH mixture is to use the Whole Mixture Model (WMM) published by the OMOE in February 1997. The WMM estimates the risk of the PAH fraction of a mixture based on the estimated exposure of Benzo[*a*]pyrene (B[*a*]P) and the cancer potency of Benzo[*a*]pyrene Surrogate (B[*a*]PS), which has a human cancer potency of 16 times B[*a*]P. OMOE states that this model can be used, and should be used, provided that it can be shown that the ratio of the total cancer potency for the PAH test mixture to the total cancer potency of the mixture of standard composition (OMOE) is between 0.1 and 10. Estimating the human health risk of a PAH mixture using the WMM has several advantages over using the IPM. The WMM is based on epidemiological human health data of exposure to PAH rich coke oven emissions where the main route of exposure was inhalation whereas the IPM uses exposure limits that are based on animal data and extrapolation to humans. Secondly, because of the derivation of the cancer potency from a mixture for mixtures, the WMM addresses the interaction of PAH compounds.

The WMM was applied to estimate the human health risk for a PAH test mixture at a contaminated former wood preserving plant. The test mixture had a total risk ratio of 1, so the human health risk was estimated based on the exposure of B[*a*]P and the cancer potency of B[*a*]PS as described in OMOE 1997. Using this procedure, inhalation, oral and dermal exposure ratios (ERs) were calculated for parkland and commercial land uses. The estimated dermal ER for the parkland use (61.2), the estimated dermal ER for the commercial use (49.7) and lastly, the inhalation ER for commercial land use (2.77) were the estimated limiting risk factors for the PAH mixture. These translated to remedial objectives of 1.44, 1.77 and 30.9 mg/kg for the subsurface soil. The OMOE estimates that the WMM offers 1-2 orders of magnitude more safety than the

IPM. The estimation of human health risk using WMM appears to offer several advantages to using the IPM, however, our results indicate that there is also one apparent disadvantage of the WMM. Human health risk estimates associated with dermal exposure to a PAH mixture appear to overestimated. It is generally accepted that, for humans, dermal exposure is the least important route, however, for the WMM, the estimate of dermal risk is based on extrapolation of human inhalation cancer potencies to oral and dermal cancer potencies. Furthermore, this extrapolation is based on extrapolation factors derived by comparing cancer potencies for inhalation, oral and dermal exposure for animals, which are more sensitive to dermal exposure than humans. OMOE estimates the level of uncertainty for this extrapolation to be within two orders of magnitude.

Human Health Risk Assessment and Management of a Tetrachloroethylene Contaminated Site. W. Ratliffe[1], M. Heise[1], M. Clendenan[1] and R. Rogers[2]. [1]EBA Engineering Consultants Ltd. (EBA), Edmonton, AB; [2]Toxcon Health Sciences Research Centre Inc.

A detailed site characterization program was conducted at an industrial site and associated landfill in northeast Alberta. Past operating and waste management practices were found to have resulted in extensive contamination by tetrachloroethylene (TCE). At the industrial site, high concentrations of TCE were present in soil, extending 35 m in depth. Shallow groundwater was also impacted with migration towards a nearby wetland occurring. At the landfill, TCE was widespread laterally, but restricted to the upper 5 m of soil. Groundwater contamination did not extend off-site. A risk-based approach to site management was taken. A human health risk assessment determined that workers at the industrial site may be exposed to unacceptable risks. At the landfill, no existing human health risks were identified, but a potential for unacceptable future risks (based on the intended land use) was found. Ecological risks were assessed through a combination of exposure assessment and direct measurement of ground and surface water toxicity. A limited potential for impact was found. The potential for natural attenuation and intrusive bioremediation to reduce the risks was investigated, but found to be insufficient. A risk management program involving limited excavation, soil treatment, and the installation of low permeability barriers was found to be the most feasible method of achieving the site specific risk management criteria.

Human Health and Ecological Risk Assessment in the Vicinity of a Crude Oil Pipeline System. M.H. Mah-Paulson, L.E. Deibert and D.R. Williams. O'Connor Associates Environmental Inc., Calgary, AB.

Abstract
A site-specific human health and ecological risk assessment was conducted for a former spill site along a crude oil pipeline system. Intrusive investigations conducted in the spill area indicated that some of the measured peat and groundwater hydrocarbon concentrations exceeded available numerical criteria. Since these criteria did not consider any site-specific factors (e.g., boreal wetland setting), a site-specific risk assessment was recommended to assess the present risks to the environment. If required, risk-based criteria would be developed that would be appropriate for the onsite peat deposits and shallow groundwater beneath the site, based on consideration of the surrounding land use and relevant receptors. The primary potential contaminants of concern to human and ecological receptors were identified to be the monoaromatic hydrocarbons

(BTEX), certain polycyclic aromatic hydrocarbons (PAHs) and the mixture as a whole (represented by total extractable hydrocarbons). The risks to relevant human and ecological receptors were assessed using quantitative exposure calculations, comparative criteria (where appropriate), toxicity testing, and a vegetation survey. The results of the risk assessment indicated that the risks associated with the identified contaminants in the peat deposits and groundwater are within ranges generally considered acceptable for the receptors and exposure pathways identified. The establishment of site-specific risk-based criteria was therefore not required. The risk assessment demonstrates the use of a combination of qualitative and quantitative approaches, appropriate to the contaminant types, exposure pathways and availability of applicable toxicity and receptor data.

Introduction

An initial site investigation by O'Connor Associates Environmental Inc. (O'Connor Associates) was conducted in response to a concern of the landowner regarding a former crude oil spill site which is located in a low lying marsh on his property. The source of the contamination is believed to have resulted from crude oil spills that occurred in the area over 25 years ago. Given the present thriving ecological system of the creek and marsh area, immediate remediation was not initiated since the net environmental benefit was not apparent, given the disturbance which may be caused by aggressive remediation techniques.

The purposes of the risk assessment were: to identify the relevant human and ecological receptors at the site; to evaluate the risks to the identified receptors associated with the contaminants of concern in the peat deposits and groundwater; and to identify the need for risk management measures, if required. The objectives of a risk-based approach are: to ensure a healthy, functioning ecosystem capable of sustaining current and likely future uses of the site by humans and ecological receptors, and to ensure that any proposed mitigative measures have a positive net environmental benefit.

Background Information

The site is located within a marsh and bog area on the south side of a creek that drains toward a lake situated approximately 4 km to the southeast. The ground surface at the site slopes to the northeast toward the creek. The area is primarily wetland with tall grasses; some shrubs and trees are located within the area on the east side of a north-south gravel road which divides the area of concern.

The principal direction of groundwater flow beneath the area is expected to be east to northeast toward the creek. The area has high, near surface, groundwater conditions. The peat is primarily fully saturated, with the exception of approximately 0.15 m to 0.3 m of the near surface material which is seasonally unsaturated. Several pipeline rights-of-way transect the area in a northeasterly direction and one pipeline right-of-way transects the area in a southeasterly direction.

The nearest groundwater wells are located at the landowner's residence situated on the north side of the creek approximately 100 m north of the area of concern. These water wells are reportedly completed to depths exceeding 20 m. Available historical information for the site indicates that the hydrocarbon contamination likely resulted from crude oil spills that occurred in the area in 1962 and 1971. It is our understanding that the spill was subjected to some remediation by burning of the residual crude oil in 1972.

Results of Intrusive Investigations
O'Connor Associates has conducted several intrusive investigations in this area since 1995. Site investigation activities have included a shallow probe hole survey, manual test hole sampling at several locations within the impacted area, sampling of surface water from the adjacent creek, sampling of the landowner's water wells, and the installation of drive-point piezometers in the surficial peat and underlying mineral soil to allow recovery of groundwater samples. The surficial soil profile underlying the area of concern consists of approximately 0.5 m to 1.5 m of peat deposits and organic-rich mineral soils overlying clay containing variable amounts of silt and sand. The peat layer is overlain by a 0.15 m thick fibrous root mat.

The results of the peat and mineral soil analyses were compared with the CCME soil quality guidelines for agricultural land use (CCME, 1991 and 1997) and the draft Alberta Tier I criteria for contaminated soil assessment and remediation. Measured peat concentrations of hydrocarbon constituents (benzene, ethylbenzene, xylenes, benzo[*b*]fluoranthene, fluorene, naphthalene and phenanthrene) exceeded the comparative criteria. The total extractable hydrocarbons concentrations were elevated with respect to the Tier I mineral oil and grease criterion in several of the peat samples.

The results of chemical analyses conducted on groundwater samples recovered from test holes and drive-point piezometers within the impacted area indicated that, at some locations, the dissolved hydrocarbon concentrations exceeded drinking water quality guidelines and freshwater aquatic life criteria. However, the results of chemical analyses conducted on groundwater samples recovered from the nearest water wells did not contain hydrocarbon concentrations exceeding the Canadian Drinking Water Quality Guidelines (Health Canada, 1996). In addition, the results of chemical analyses conducted on surface water samples recovered from the adjacent creek did not contain hydrocarbon concentrations exceeding freshwater aquatic life criteria (CCME, 1987/1996).

Remediation Options
Subsequent to characterization of the petroleum-impacted peat deposits, potential options for the treatment and/or disposal of the impacted peat were reviewed. These included: intrinsic bioremediation; *in situ* bioremediation; *ex situ* bioremediation; excavation and landfill disposal; incineration; and thermal desorption/adsorption.

The extent of impacted peat was estimated based on the measured peat and groundwater concentrations which exceeded comparative criteria. However, the comparative soil criteria did not consider any site-specific factors (e.g., boreal wetland setting) and, as the comparative soil criteria are for typical mineral soils, they were not considered applicable for the high organic-rich peat materials at the site. A site-specific risk assessment was therefore recommended to assess the present risks to the environment and if required, to develop risk-based criteria that would be appropriate for the onsite peat deposits and shallow groundwater beneath the site, based on consideration of the surrounding land use and relevant receptors.

Based on the comparative criteria, the estimated volume of impacted peat was relatively large (approximately 11,000 m^3). Therefore, it would be advantageous to develop site-specific, risk-based, clean-up criteria, such as equivalent Tier I criteria for peat, to minimize the volume of impacted peat requiring remediation. In addition, a site-specific risk assessment would also evaluate the net environmental benefit of aggressive remediation versus the thriving ecological system observed in the creek and marsh area. There are different remediation approaches and various potential options available to address the petroleum-impacted peat deposits. Ultimately, the most appropriate approach and option(s) must balance the requirements and objectives of all stakeholders, including the landowner and the regulators.

Risk Assessment

The risk assessment methodology used herein is consistent with relevant guidance documents and protocols published by Health Canada, Canadian Council of Ministers for the Environment (CCME) and the United States Environmental Protection Agency (US EPA). The basic steps in the risk assessment process are: problem formulation; exposure assessment; hazard assessment; and risk characterization.

Contaminants of Concern

Crude oil is a complex mixture of paraffinic, cycloparaffinic (naphthenic) and aromatic hydrocarbons. The most common aromatic compounds in crude oil include benzene, benzene derivatives and fused benzene ring compounds. A review of relevant literature and US EPA toxicological databases (IRIS and HEAST) was conducted to identify compounds within crude oil which may be considered potential contaminants of concern to human and ecological receptors. On the basis of the site data and analytical results obtained to date, as well as the literature review, the primary potential contaminants of concern to human and ecological receptors were considered to be the monoaromatic hydrocarbons benzene, toluene, ethylbenzene and xylenes (BTEX), certain polycyclic aromatic hydrocarbons (PAHs) including benzo[*b*]fluoranthene, fluorene, naphthalene and phenanthrene, and the mixture as a whole (represented by total extractable hydrocarbons).

Human Receptors and Exposure Pathways

The impacted area is accessible to the landowner's cattle but, due to the soft wetland conditions and the numerous active pipelines in the immediate area, human usage is infrequent. However, the potential for human exposure exists and was therefore included in this assessment. Land use in the vicinity of the site is predominantly oil and gas related and agriculture (cattle grazing). The nearest potential human receptors in the area are occupants of the landowner's residence and occasional recreational users of the land.

Future residential occupants of the land were also identified as potential receptors. However, due to the physical site setting, in particular the high water table and presence of peat, it is unlikely that future residential development would occur in the wetland area (particularly in the impacted area). Potential exposure pathways include: ingestion of groundwater; direct skin contact with contaminated peat and/or groundwater; incidental ingestion of contaminated peat and/or groundwater; inhalation of suspended particulate matter containing contaminants; ingestion of wildlife/livestock in which contaminants may have bioaccumulated; dermal contact with contaminated surface water; and incidental ingestion of contaminated surface water.

Ecological Receptors and Exposure Pathways

The site is located in an ecoregion characterized by aspen, spruce, willow and sedges. The immediate site area is primarily wetland with tall grasses. Cattle have access to this area. The selected ecological receptors considered are: mammals/birds including cattle, deer, beaver, meadow voles, red-tailed hawk and mallard; vegetation; invertebrates and microbial processes; and aquatic species (aquatic plants, microscopic and macroscopic aquatic animals).

The identified ecological receptors could be exposed to the potential contaminants of concern via several pathways including: consumption of surface water by cattle; consumption of water by other mammals/birds; uptake by vegetation; direct contact (dermal and ingestion) with contaminated peat by terrestrial animals and invertebrates; bioaccumulation and food chain entry; and direct exposure to surface water by aquatic species.

Hazard Assessment/Risk Characterization
The overall approach used in the hazard assessment and risk characterization stages comprised a combination of quantitative exposure calculations, a qualitative assessment of the vegetation, comparison with relevant criteria, and toxicity testing.

Quantitative Analyses
Quantitative analyses were conducted to evaluate the risks associated with the identified contaminants in the peat deposits using toxicological information available through US EPA sources such as IRIS and HEAST. Doses were estimated and risks/hazards to human receptors were evaluated using cancer slope factors for carcinogenic compounds and reference doses for non-carcinogenic (threshold) compounds. Estimated average daily doses for the mammals and birds were compared with published NOAEL (no observed adverse effects level) and LOAEL (lowest observed adverse effects level) data.

Based on the maximum hydrocarbon concentrations measured in the impacted peat samples, the predicted exposures (due to direct and indirect soil exposure pathways) indicated no exceedances of the target risk of $1x10^{-5}$ or the target hazard index of 0.2 for human receptors. Based on the maximum hydrocarbon concentrations measured in the impacted peat samples and surface water samples, the predicted maximum exposures to mammals and birds did not exceed the allowable daily threshold effects doses for the ingestion of peat, food, and surface water.

Vegetation Survey
The purpose of the vegetation survey was to compare the abundance and health of indigenous vegetation in impacted and unimpacted (control) areas of the site. Three impacted plots and two reference (control) plots were identified for the survey. The impacted and reference plots contained similar species and plant vigour. The plant species identified were typical of those found in wetland settings. The results of the vegetation survey indicated that, overall, there were no significant differences in vegetation between the impacted and reference plots.

Criteria Approach
The criteria approach was used in evaluating the following exposure pathways: ingestion of groundwater as potable water (human receptors); and direct exposure to surface water by aquatic species. In evaluating the risks associated with the ingestion of groundwater as potable water, the concentrations of the identified contaminants of concern measured at the point of exposure (i.e., the nearest water wells) were compared with the Canadian Drinking Water Quality Guidelines (Health Canada, 1996). To date, the measured concentrations at the nearest water wells have been less than the comparative criteria. Some groundwater concentrations measured within the impacted area have exceeded the comparative criteria. However, based on the distance to the nearest water wells (100 m north of the impacted area), the high absorptive capacity of the peat, the hydraulic barrier created by the adjacent creek (located between the water wells and the impacted area) and the analytical data at the water wells, there was no expected impact on the nearest water wells. The likelihood of impact on future water wells

potentially located closer to the impacted area was also low based on the high absorptive capacity of the peat and the probable depths of screen placement beneath the shallow impacted peat.

In evaluating the risks to aquatic species in the adjacent creek, the concentrations of the identified contaminants of concern measured at the point of exposure (i.e. the adjacent creek) were compared with freshwater aquatic life criteria published by the CCME (1987/1996). To date, concentrations of the contaminants of concern have not been detected in surface water samples recovered from the adjacent creek. Some groundwater concentrations measured within the impacted area have exceeded the comparative criteria. However, based on the absorptive capacity of the peat and the analytical data at the creek, there was no expected impact at the creek adjacent to the spill site.

Toxicity Testing

The objective of the toxicity testing was to establish the hydrocarbon concentrations in the peat deposits which would result in a toxic response in organisms which thrive in the wetland. Peat samples were recovered from impacted and unimpacted areas for toxicity testing. The impacted peat sample used in the toxicity testing was recovered from the mostly highly impacted area of the site.

The toxicity tests comprised of: an algal growth inhibition test with *Selenastrum capricornutum*; and survival and reproduction test with *Ceriodaphnia dubia*. These tests were considered appropriate for the determination of whether the hydrocarbon impact in the most highly affected peat was toxic to organisms dwelling in the wetland. The tests were conducted following Environment Canada test methods (Environment Canada, 1992a, 1992b). *C. dubia* is considered to be one of the most sensitive of the standard toxicity tests available.

The results of the toxicity testing indicated that the peat samples recovered from both the impacted and unimpacted areas were not toxic. Therefore, the hydrocarbon contaminated peat at the site was not expected to pose any unacceptable risks to invertebrates and microbial processes.

Summary and Conclusions

A site-specific risk assessment has been conducted for receptors at a former crude oil spill site. Although the likelihood of direct human exposure at the site was considered low, a human health risk assessment was conducted which considered the potential for human receptors in the vicinity to be exposed to the identified contaminants of concern. Future residential development on or near the impacted area was considered unlikely based on the physical site setting. Critical ecological receptors at the site were identified to be mammals/birds, vegetation, aquatic species, and invertebrates and microbial processes.

Risks were evaluated using quantitative assessments of exposure, qualitative assessment of toxic effects (vegetation survey), comparison with relevant applicable criteria, and toxicity testing. The results of the risk assessment indicated that the risks associated with the identified contaminants of concern in the peat deposits and groundwater were within ranges generally considered acceptable. The establishment of site-specific risk-based criteria was therefore not required.

Risk assessment does not remove the requirements for risk management. Risk management measures at the site include ongoing sampling of groundwater from the drive-point piezometers within the impacted area to ensure that dissolved concentrations of the identified contaminants of concern remain stable and/or are decreasing with time. Routine sampling of the nearest water wells and the adjacent creek are also ongoing. Continued monitoring of subsurface conditions allows for the verification of assumptions made in conducting the risk assessment. The goals of risk management are receptor protection, exposure prevention and subsequent risk reduction. Therefore, localized excavation of "hot spots" was also proposed. Risk assessment is a risk management tool, and as with any form of risk management, requires stakeholder agreement on the risk management objectives.

References

Alberta Environmental Protection. 1994. Alberta Tier I Criteria for Contaminated Soil Assessment and Remediation. (Draft).

Canadian Council of Ministers of the Environment (CCME). 1997. Recommended Canadian Soil Quality Guidelines. March.

Canadian Council of Ministers of the Environment (CCME). 1991. Interim Canadian Environmental Quality Guidelines for Contaminated Sites. September.

Canadian Council of Ministers of the Environment (CCME). 1987 (updated 1996). Canadian Water Quality Guidelines.

Environment Canada. 1992a. Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*. Report EPS 1/RM/21.

Environment Canada. 1992b. Biological Test Method: Growth Inhibition Test Using the Freshwater Alga Selenastrum capricornutum. Report EPS 1/RM/25.

Health Canada. 1996. Guidelines for Canadian Drinking Water Quality. Prepared by the Federal-Provincial Subcommittee on Drinking Water of the Federal-Provincial Committee on Environmental and Occupational Health. Sixth Edition.

United States Environmental Protection Agency (US EPA). 1999. Integrated Risk Information System (IRIS). On-line.

United States Environmental Protection Agency (US EPA). 1997. Health Effects Assessment Summary Tables (HEAST), FY 1997 Update. Office of Solid Waste and Emergency Response, Washington, D.C., EPA/540/R-94/020. July.

Accidental Release of PCBs, Dioxins and Furans from the Swan Hills Treatment Centre: Assessment of Potential Human Health Risks via Game and Fish Consumption. R. Compton[1], G.L. Brown[1], G. Latonas[2]. [1]Cantox Environmental Inc., Calgary, AB; [2]BOVAR Waste Management, Calgary, AB.

An accidental release in October 1996 of PCBs, dioxins and furans at the Swan Hills Treatment Centre (SHTC) received considerable attention due to the potential health risks and environmental impacts. Subsequent to the release, Alberta Health issued a Public Health Advisory limiting consumption of game and fish within a 30 km radius of the SHTC. The advisory was based on the analysis of game and fish samples collected in the vicinity soon after the release and the results of a consumption survey of Swan Hills residents. Since this time, considerable data collection and study has taken place which has bearing on the public health advisory and has helped to define and quantify the health risks resulting from the release.

The primary human receptors (i.e., people of concern) were considered to be hunters and their families exposed via wild game consumption and people consuming fish from Chrystina Lake. Risks to human health were characterized by comparing potential daily intake of PCBs and 2,3,7,8-TCDD toxic equivalents (TEQ) via game and fish consumption with the tolerable daily intakes (TDI) for PCBs and dioxin recommended by Health Canada. For exposure calculations regarding consumption of wild game and fish, the results of the consumption survey of Swan Hills residents by Alberta Health (1997) were assumed. Based on the reported consumption rates, four consumption categories were identified for game and fish eaters (i.e., high, medium, low and very low intake).

The Alberta public health advisory was based on game samples of deer and moose collected between October 1996 and February 1997, as well as fish samples collected in June 1997 from Chrystina Lake (1.5 km from the SHTC) and Roche Lake (20 km from the SHTC). The advisory recommended limiting consumption of game taken from a 30 km radius of the SHTC to 370 g per month and limiting consumption of fish from within a 20 km radius to 170 g per week. Pregnant or nursing mothers and small children were advised to avoid eating game or fish from these areas.

Alberta Health used essentially the same approach for establishing the advisory as described in the current risk assessment (i.e., calculating exposure ratios based on the chemical concentration, consumption rate, body weight and tolerable daily intake). Based on the data available at the time, their assessment was conservative but not unreasonable. However, measured chemical concentrations in game animals analysed in the Fall of 1997 and 1998 by BOVAR and in early 1999 by Alberta Health indicate that the concentrations of PCBs and PCB/PCDD/F TEQ in game near the SHTC have decreased considerably since the Fall of 1996 when Alberta Health analysed the original game samples collected near the SHTC. Risk assessment indicates that even for high consumers of game meat (i.e., on a daily basis), potential exposures to these compounds through consumption of game since Fall, 1997 were substantially less than the exposure limits recommended by Health Canada.

The Alberta Health data included reference samples and it provides evidence that the study area samples have higher concentrations of PCBs and PCB/PCDD/F TEQ relative to samples from control areas. This is not unexpected since even under normal operations some level of emissions occur on an ongoing basis. However, the concentrations of PCDD/F TEQ and PCBs measured in the Fall 1997 and 1998 game samples were similar to those reported in beef, pork and veal samples from Canadian supermarkets.

Model predictions of potential concentrations of PCBs and TEQ in moose in the vicinity of the SHTC were also made. The model predictions were higher than the measured concentrations, likely due to conservative assumptions incorporated into the model. Risk assessment based on the modelled concentrations indicates that if game species foraged continuously in the immediate vicinity of the SHTC (i.e., within 2.6 km), their body burdens could pose potential health risks for high game meat consumers. However, when it was assumed that the moose had not spent any time within 1 km of the SHTC, no health risks were predicted for any consumer. The possibility of harvesting a moose (or deer) that lived continuously very close to the SHTC is considered low.

The fish consumption risk assessment was based on historically measured maximum concentrations in Chrystina Lake fish each year from 1995 to 1998, in addition to modeled concentrations. The predicted concentrations for brook trout were in close agreement with measured concentrations in Chrystina Lake fish sampled in June, 1998. BOVAR also collects samples from Victor Lake, located approximately 260 km from the SHTC, which is considered a control lake.

The measured and modeled total PCB concentrations in Chrystina Lake fish were significantly below the Canadian guideline for PCB concentrations in fish on an edible portion basis, and generally fell within the range of values determined in freshwater fish from other lakes throughout Canada. The risk assessment indicates that exposure to PCBs through the consumption of white suckers and brook trout from Chrystina Lake would not exceed the tolerable daily intake established by Health Canada. Fish collected from Victor Lake were found to have non-detectable concentrations of total PCBs.

Human exposure to total TEQ measured in sampled Chrystina Lake fish was estimated to slightly exceed the tolerable daily intake (TDI) established by Health Canada (1996). Exposure ratio (ER) values of 1.1 to 1.6 were calculated for fish sampled from 1995 through 1998 (ER = exposure/exposure limit). However, this is true only for high fish consumers (167 g/d) and is based on the conservative assumption that 100% of consumed fish would come from Chrystina Lake, which is unlikely given the size of the lake.

The TEQ concentrations measured or modeled in fish from Chrystina Lake were higher than the concentrations determined in fish from Victor Lake or the hatchery. However, the PCDD/F TEQ concentrations are within the range of concentrations measured in fish from the Great Lakes and the PCB-TEQ concentrations are within background concentrations in fish from the Canadian Arctic. In addition, neither the PCDD/F TEQ concentrations or the calculated total TEQ concentrations (i.e., PCDD/F TEQ and PCB TEQ) exceed the Health Canada 20 ng/kg tolerance guideline for TCDD in fish tissue, nor the provisional guideline of 15 ng/kg.

A Weight-Of-Evidence Approach as Part of Northern River Risk Assessment: Integrating the Effects of Multiple Stressors. R.B. Lowell and J.M. Culp. Environment Canada, National Water Research Institute, Saskatoon, SK.

Northern river ecosystems are subject to a variety of stressors having multifaceted (and sometimes opposing) effects, making interpretation at a regional scale difficult. We have addressed this problem by using a weight-of-evidence approach that combines analysis of field data (to determine patterns) with experimental hypothesis testing (to determine mechanisms). Two of the more important sources of aquatic impacts in western Canada are pulp mill and municipal effluents. Their regional impacts on benthic biota were evaluated for two major river systems, the Thompson and Athabasca Rivers, using an integrative approach. In the more southerly Thompson River, several lines of evidence (including field and laboratory experiments, field sampling over a 20 yr period, and isotopic analysis) led to the conclusion that, while some toxic effects were apparent, these effects were usually masked by the (sometimes excessive) nutrient enhancement effects of these effluents, sometimes via novel pathways. In the more northerly Athabasca River, effluent effects can be modified by the added impact of another stressor, widespread winter freeze-up which prevents reaeration of oxygen depleted waters, coupled with low dissolved oxygen levels in the substratum where benthic invertebrates are found, resulting in a net shift in effluent effect from one of nutrient enhancement to a more inhibitory effect. Advantages to applying formalized causal criteria, as outlined in this weight-of-evidence approach, include helping to tie together diverse assemblages of data on the effects of multiple stressors, and identifying important informational gaps, thus making ecological risk assessments more rigorous and robust.

Calculating and Applying EC*p* and its Confidence Intervals (CI) in Ecological Risk Assessment (ERA). M.D. Paine. Paine, Ledge and Associates (PLA), North Vancouver, BC.

EC*p* are concentrations at which effects of *p*%, relative to field references, laboratory controls, or some other standard, occur. EC*p* are estimates of *x* (concentration) for some value of *y* corresponding to *p*% effects, and are derived from dose-response relationships. The width of CI for EC*p* depends on *n*, the number of contaminated or "impact" samples used to estimate the dose-response relationship, and *m*, the number of samples used to estimate the reference or control response. The optimal sampling allocation is $n \approx 2m$, although $n \leq 5m$ will usually be adequate. If effects are expressed relative to a fixed standard, such as control standards for a toxicity test, $m = \infty$. If effects are expressed relative to a laboratory control tested simultaneously with the impact samples, $m = 1$, regardless of the number of laboratory control "pseudoreplicates" tested. In ERA, EC*p* can be used to classify samples or sites as acceptable/unacceptable, or as remediation criteria. CI can be used to estimate the uncertainty about estimates of EC*p*. However, the author is philosophically opposed to using upper or lower confidence limits (CL) for EC*p* as decision criteria. CL for EC*p* are just EC for some other *p*, and depend on variances, *n*, and *m*. To calculate EC*p*, you start with some effect size (*p*) you think is important. Why let the vagaries of study design alter that *p*?

Assessment of the Toxicity and Interaction of Pesticide Mixtures using a Combination Approach of Probabilistic Risk Assessment and Toxic Equivalents. T.K. George[1], K. Liber[1], K.R. Solomon[2] and P.K. Sibley[2]. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; [2]Centre for Toxicology, University of Guelph, Guelph, ON.

The presence of chemical mixtures in aquatic systems has increased the demand for risk assessment approaches that can accommodate mixture analysis. Under investigation, is a combination of probabilistic risk assessment (PRA) and toxic equivalents (TE). To study this approach, pesticide mixtures were applied to freshwater microcosms and the response of zooplankton populations assessed. The first mixture examined was a binary combination of organophosphate insecticides diazinon and chlorpyrifos, and the second, a ternary mixture of three different types of pesticides, chlorpyrifos, endosulfan and trifluralin. The potential interaction of these mixtures was investigated by preparing both mixtures in different proportions, but theoretically of equal potency. Each mixture was dominated by one pesticide (≥80%) and remaining pesticides were apportioned equally (~10%). To achieve equal toxicity, toxic equivalents were used and components of the mixtures were normalized to the most toxic component, chlorpyrifos. Results of the binary organophosphate mixture study showed that zooplankton populations responded similarly to each mixture, confirming equitoxicity. Conversely, the toxicity of the ternary mixtures to the major zooplankton taxa was reflective of the susceptibility of each taxon to the dominating pesticide of the mixture. The location of taxa within the sensitivity distribution for each pesticide differed, and susceptible taxa near the 10^{th} centile displayed a significantly greater toxic response to mixtures dominated by that pesticide. The PRA-TE approach should be limited to mixtures of pesticides with similar modes of action and does not appear to be suitable to assess pesticide mixtures with different modes of action.

Application of a "Top-Down" Approach in Ecological Risk Assessment: An Alternative to the Hazard Quotient Fixation. S.M. Swanson, L. Mucklow, D. Kerr, M. Raine and V. Chisholm. Golder Associates Ltd., Calgary, AB.

The traditional "bottom-up" approach to ecological risk assessment for contaminated sites involves the following steps: determine chemical concentrations in environmental media; determine toxicity thresholds from the ecotoxicological literature; calculate hazard quotients for the receptors of concern; and use hazard quotients as the basis for remediation decisions. This approach is focused on chemical concentrations and modelling to extrapolate up to possible effects on the receptors of concern. An alternative "top-down" approach involves evaluating differences in ecosystem health of populations and communities between contaminated and reference areas. This approach is consistent with recent developments in expanding traditional ecological risk assessments to population and community level assessments. The "top-down" approach incorporates population and community level measurements to assess ecosystem health, rather than individual level measurements determined from chemical analysis, food chain modelling and the ecotoxicological literature.

The "top-down" approach uses the ecosystem as the overall context for making decisions about remediation of contaminated areas. This approach avoids the problems with starting at the bottom (chemical concentrations) and attempting to simulate complex processes to predict risk (i.e., bioavailability, chemical form, mixtures, extrapolating from laboratory toxicity data to the field). The ecosystem health approach starts from the top and concentrates on the communities and populations that have already integrated their response to the chemicals within their environment. It uses a weight of evidence approach as the basis for remediation decisions. The key assumptions inherent in the "top-down" approach are: ecosystem responses can be measured by field techniques; measurement of community and population responses provides more reliable, site-specific information than extrapolating from chemical concentrations and the ecotoxicological literature; and removing viable habitat based on bottom-up extrapolations may cause more damage to populations of the receptors of concern.

This approach relies on a rigorous field program to identify differences in a variety of population and community measures, such as species richness, abundance, diversity, percent cover, stress symptoms and habitat suitability for the receptors of concern. It is also beneficial to collect some samples of soil, plants and small mammals for chemical analysis to aid in determining cause-effect relationships. The field data is statistically analyzed to determine whether there are significant differences in each measure between contaminated and reference areas. The data is then more rigorously analyzed to determine the potential causes of the observed effects (i.e., chemical concentrations or physical factors). Finally, statistical differences are evaluated to determine their ecological significance relative to maintenance of ecosystem health. A weight of evidence approach is used to determine which areas of contamination require remediation.

Legal Implications of Investigative Toxicology

Legal Implications of Investigative Toxicology. J. Purdy. Novartis Crop Protection Canada, Guelph, ON.

Companies involved in developing new pest control products for the Canadian market have many regulatory hurdles to overcome before launching any new product in the Canadian market. Under the *Pest Control Products Act* and Regulations, the vendor must submit proof that the new product has "merit, value and safety," before the product can be registered for use in Canada. All submitted information regulation must meet two tests of validity: First, the data must be complete (it must pass a data screening procedure and 2nd the data must pass a scientific review to ensure that all issues have been addressed. For the Safety aspect of this regulation compliance to Good laboratory Practice standards has been an industry standard in Canada for more than ten years, and starting in Jan 2000, GLP compliance will be required by law. Product introduction, already a very risky, expensive and time consuming process, will bear an added burden of legal liability. Much of the actual work is done under contract, and with this much at stake, companies are at considerable risk. External quality certification programs (ISO, CAEAL) are of limited use in assuring that the research will be acceptable, and will be superceded by the Standards council of Canada GLP Accreditation. Even this accreditation provides no more than a starting point for contractor selection. In effect, the company must take control of the selection and development of quality contract resources within its contractor selection procedures. This paper presents a review of industry experience and strategies in research to support registration of product for the Canadian market.

Good Science in not Enough. S. McRory[1] and A. Moen[2]. [1]Alberta Justice, Edmonton, AB; [2]Fraser Milner, Barristers & Solicitors, Edmonton, AB.

Abstract not available.

Collection of Evidence to Support a Prosecution Following a Condensate Spill. D.A. Birkholz[1], D. Johnston[1] and A. Bollo-Kamara[2]. [1]Enviro-Test Laboratories, Edmonton, AB; [2]Alberta Environment: Enforcement and Monitoring, Edmonton, AB.

Following a spill of condensate into the aquatic receiving environment, samples of fish bile were collected from longnose sucker (*Catostomus catostomus*) and rainbow trout (*Oncorhynchus mykiss*) upstream and downstream of the spill site. Bile samples were composited for each species and enzymatically hydrolyzed, extracted, acetylated, silylated and analyzed using gas chromatography/mass spectrometry. Acetylation prior to GC/MS analysis, generated unique mass spectra which allowed one to distinguish alcohol and phenol metabolites from one another. Silylation prior to GC/MS analysis does not allow one to distinguish phenol from alcohol metabolites. The major metabolites found to be present in the bile of fish collected downstream of the spill site included alcohol and phenol metabolites associated with exposure to C_2-C_4 substituted naphthalene, phenanthrene, fluorene and dibenzothiophene. These compounds were found to be present in the spilled condensate. The highest concentration of metabolites found in the bile of exposed fish were observed to be alcohols associated with exposure to C_2-substituted dibenzothiophene and fluorene. It is suggested that these metabolites be monitored to determine whether cleanup efforts are sufficient to reduce future exposure. Bile analyzed from fish taken upstream of the spill site did not contain any of the metabolites found in the exposed fish. This information was collected to determine the potential impact of the spill on the aquatic receiving environment.

Deposit, Aquatic Fate and Short-term Effects of Trichlorfen after Aerial Forestry Applications in Newfoundland. G.R. Julien[1], M.A. Savard[1], W.R. Ernst[1], D. McCall[1], K.G. Doe[2], J. Banoub[3] and P. Jackman[2]. [1]Environment Canada, Environmental Protection, Dartmouth, NS; [2]Environment Canada, Environmental Conservation Branch, Moncton, NB; [3]Department of Fisheries and Oceans, St. John's, NF.

An aerial pesticide spray program using Dylox® (750 g trichlorfon/ha) was conducted in July 1998 to combat the balsam fir sawfly (*Neodiprion abietis*). A study was undertaken to determine the effectiveness of a 200 m watercourse buffer by measuring deposit on collectors, aquatic contamination and effects on invertebrates in two pond/stream systems. While the concentrations detected were over 200 times greater than the 96 h LC_{50} for stonefly cited in the literature, the duration of elevated concentrations were much shorter than those LC_{50} exposures. Some water samples from time periods up to 14 h post-spray exceeded invertebrate (*Daphnia carinata*) LC_{50} values for comparable exposure times (i.e., 3-6 h LC_{50}). All samples up to 5 h post-treatment from a poorly buffered watercourse immobilized *Daphnia magna* and lethal effects were observed during 48 h exposures. There were also reductions in numbers of phytoplankton and aquatic macroinvertebrates up to 48 h post-spray. The results and observations indicated that a 200 m watercourse buffer was inadequate to prevent deposition of trichlorfon at concentrations that pose a risk to aquatic organisms.

POSTER SESSION/SESSION D'AFFICHAGE

Petrochemical Industry Issues

Oil Sands Process Water Toxicity in Midge larvae (Diptera: Chironomidae): Evidence for Adaptation? M.P. Whelly and J.J.H. Ciborowski. Department of Biological Sciences, University of Windsor, Windsor, ON.

Wetlands that receive oil sands process water (OSPW) are enriched with several potentially toxic classes of compounds (metals, PAHs, other hydrocarbons). Yet, they support populations of midges that are apparently unaffected by these compounds. This suggests that either adaptation to contaminants has occurred in the chironomid populations, or that the toxic components in the highly organic wetlands are not bioavailable to the organisms. To address these questions, a 14 d static laboratory bioassay was conducted using first-instar larvae of two laboratory (*Chironomus riparius* and *C. tentans*) and one field population (*C. tentans*) exposed to OSPW. Survival and growth were significantly reduced for both lab (at 50 and 100% OSPW) and field *C. tentans* (100% OSPW). Chironomid larval development was slowed but not arrested, as reflected by reduced body lengths for the field and particularly the lab *C. tentans*, and reduced emergence rates for *C. riparius* at 100% OSPW. Incidence of chironomid mouthpart deformities (extra or missing teeth) was used as a teratogenicity biomarker. However, no significantly increased incidences were observed in relation to OSPW exposure. Deformity levels ranged from 1.2-3.6%, 3.3-5.1%, and 1.3-17.1%, for field *C. tentans*, lab *C. tentans*, and (lab) *C. riparius*, respectively. Differences in population sensitivity based on growth and survival suggest that field adaptation to OSPW compounds has occurred.

Toxicological Studies on Polycyclic Aromatic Compounds of Importance to Oil Sands Reclamation. B.G. Brownlee[1], G.A. MacInnis[1], R.E.A. Madill[2], M.T. Orzechowski[2] and N.J. Bunce[2]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Department of Chemistry and Biochemistry, University of Guelph, Guelph, ON.

Near Fort McMurray in northeastern Alberta, several hundred thousand tonnes of oil sands per day are extracted by the Clark hot water process to produce crude bitumen for production of synthetic crude oil. Tailings, containing water, sand, fines and small amounts of unextracted bitumen are pumped to large above ground ponds. Coarser particles settle out quickly to form beach material, the fines slowly consolidate and settle in the open water area of the ponds, eventually forming "mature fine tails" (MFT). One method of long term management of MFT for site reclamation is transfer to old mine pits which are below ground and capping them with clean water. We have been studying the toxicology of MFT interstitial water by testing extracts and representative polycyclic aromatic compounds (PACs) identified therein. The Ames *Salmonella* test was the most easily interpreted of the test methods used and indicated a very low mutagenic potential for the PACs in MFT interstitial water [Madill *et al.* Environ. Sci. Technol. (in press)]. Twenty-one individual PACs were tested for mutagenicity by the Mutatox® test using both the direct (without S9) and indirect (with S9) procedures. Three- and four-ring PACs, consisting of parent and methylated PAHs, heterocyclic (N and S) analogs, and oxidized derivatives (hydroxy-PAHs and sulfones) were tested. In general, non-oxygenated PACs were inactive or weakly active with S9 and more strongly active without S9 activation. Oxygenated derivatives tended to be inactive both with and without S9. The murine Ah receptor assay [Schneider *et al.* Environ. Sci. Technol. 29:2595-2602 (1995)], adapted to use benzo[*a*]pyrene (B[*a*]P) as the reference radioligand, was applied to a subset of these PACs. The range of relative binding affinities (RBAs), relative to 10 nM B[*a*]P, was less than one order of magnitude for the PACs studied, namely 0.003 to 0.02. RBAs correlated weakly with the octanol-water partition coefficient for these compounds, suggesting that factors besides lipophilicity are also important in determining the strength of binding for PACs.

The Effects of Petroleum Exposure on the Feeding Behaviour of Rainbow Trout (*Oncorhynchus mykiss*) Using the Water-soluble Fraction of Norman Wells Crude Oil. M.J. Ryan[1] and W.L. Lockhart[2]. [1]Department of Zoology, University of Manitoba, Winnipeg, MB; [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

The effects of exposure to the water-soluble fraction (WSF) of Norman Wells crude oil (NWC) on the feeding behaviour of rainbow trout were studied. Trout were exposed to WSF of NWC mixed at 10, 25, 50, 100, 200 and 400 mg/L nominal concentrations. Exposures lasted 14 d with complete, daily renewal of the WSF in a static, non-aerated system. Nominal hydrocarbon (HC) concentrations were compared with measured concentrations by extracting water with hexane and analyzing the samples with mass spectroscopy gas chromatography (GC-MS). At 400 mg/L nominal (8.27 mg/L measured) HC concentrations, all the fish died within 96 h. Between 50 mg/L and 200 mg/L nominal concentrations (0.55-1.92 mg/L measured concentrations), feeding became noticeably slower within 2 d and stopped completely by 3 d. The fish displayed signs of narcosis and swimming impairment with >92% decrease in daily food consumption. The "lowest observable effect concentration" (LOEC) was 25 mg/L nominal (0.31 mg/L measured) HC concentrations. Feeding slowed after 5 d resulting in a decrease in food consumption >54% after 14 d when compared to controls. No effects on feeding behaviour were evident at the "no observable effect concentration" (NOEC) of 10 mg/L nominal (0.14 mg/L measured) HC concentrations. The maximum allowable toxicant concentration (MATC) for the alteration of feeding behaviour in young rainbow trout ranged between NOEC and LOEC at 0.14 to 0.31 mg measured HC per litre of water. There was a small, but statistically insignificant increase in body water contents ($p=0.07$, ANOVA). The study shows that the WSF of crude oils adversely affects the feeding behaviour of rainbow trout at concentrations that can be found in contaminated areas.

The Mammalian Toxicity of Naphthenic Acids Derived from Athabasca Oil Sands. V.V. Rogers[1], M. Wickstrom[1], M.D. MacKinnon[2] and K. Liber[1]. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; [2]Syncrude Canada Ltd., Edmonton, AB.

Naphthenic acids are a diverse group of petroleum-associated carboxylic acids having mono- and polycyclic alkane bodies and aliphatic side chains of various lengths. These natural surfactants are the major component of Athabasca Oil Sands (AOS) process water responsible for aquatic toxicity, but little is known about their mammalian toxicity. The volume of process-affected waters retained on Syncrude's present lease alone is expected to exceed $10^9 m^3$ by the year 2025, representing a source of long-term exposure to indigenous wildlife as well as to the surface aquatic environment. The objectives of the present study are to describe the mammalian toxicity of naphthenic acids derived from AOS process waters, and to determine the mechanism(s) of toxic action. Naphthenic acids, isolated from Syncrude's main holding pond (Mildred Lake Settling

Basin), were administered in single doses (0, 3, 30 and 300 mg/kg body weight) to Wistar rats, which were then examined 14 d later. Acute dosing was found to be hepatotoxic at 300 mg/kg, while significant cardiovascular effects, including colonic and cerebral haemorrhaging and myofibrosis, were elicited at concentrations as low as 30 mg/kg. Histological lesions were not apparent in renal, reproductive, pulmonary or lymphoid tissues. The highest dose also led to a decrease in food consumption and temporary weight loss. Ongoing tests include a 90 d, daily exposure study (0, 0.3, 3 and 30 mg/kg per d). Endpoints of interest will include histology, hematology and clinical chemistry, with a focus on possible mechanisms associated with the previously observed haemorrhage (clotting factor or platelet change) and hepatobiliary effects.

The Use of SPMDs and Small Sentinel Species to Assess Naturally-Occurring Oil Sands Compounds in Tributaries of the Athabasca River. J. Parrott[1], C. Portt[2], M. Baker[1] and M. Colavecchia[1]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]C. Portt & Associates, Guelph, ON.

To assess the potential effects of natural oil sands exposure, small fish species (slimy sculpin *Cottus cognatus* and trout-perch *Percopsis omiscomaycus*) were sampled from several Athabasca River tributaries upstream and downstream of the oil sands formation. As well, semipermeable membrane devices (SPMDs) were deployed for two weeks in these same locations to concentrate neutral organic compounds from river waters. Slimy sculpin showed increased mixed function oxygenase (MFO) at several downstream sites, where there was exposure to oil sands related chemicals. Highest MFO activities were in male sculpin from the downstream Ells River site, which had 10-20 fold induction compared to upstream sites on other rivers. Elevated MFOs were seen in sculpin from the lower Muskeg and Lower Steepbank Rivers. Trout-perch had much lower MFO activities than slimy sculpin, and showed no significant differences among sites. SPMDs concentrated compounds from the downstream sites, and extracts of SPMDs induced MFO activity in fish liver cell lines. The most potent natural oil sands site was the downstream Ells River site, followed by downstream Muskeg River site, then the Steepbank and MacKay Rivers. There was little or no induction in SPMD extracts from upstream sites, and no induction in trip blanks. Generally, MFO activity in livers of sculpin agreed well with MFO induction potency in fish liver cells exposed to SPMD extracts from the same sites. Since this was a preliminary site assessment year, numbers of fish captured were low as fishing time was limited to 2-3 h per site. Full fish collections will occur in the fall of 1999, with mainstem Athabasca River sites added to assess potential anthropogenic oil sands mining/refining input, and to determine the suitability of these small species for monitoring areas of the Athabasca River system.

Endocrine Disrupting Compounds

An *in vitro* Bioassay for Environmental Estrogens Based on Rainbow Trout (*Oncorhynchus mykiss*) Hepatocytes and its Use to Assess the Estrogenicity of Black Liquor and Final Effluents from the Pulping Process. J.P. Sherry, T. Hooey and N. Spitale. Environment Canada, National Water Research Institute, Burlington, ON.

The development and characterization of an *in vitro* bioassay for the detection of estrogenic activity is described. The assay is based on the induction of vitellogenin in primary cultures of rainbow trout hepatocytes. Some critical factors for the reliable production of high quality cultures of hepatocytes in good yields were identified. The induced Vg was measured by competitive enzyme linked immunosorbent assay (ELISA). 17ß-estradiol was used as a model estrogen to optimize and characterize the assay's performance. Nonylphenol and octylphenol were used to verify the assay's ability to detect known environmental estrogens. The bioassay was used to detect estrogenic activity in black liquor from the pulping process. A static renewal bioassay was used to test the black liquor's ability to induce Vg *in vivo.* The estrogenicity of some pulp mill effluents was also tested.

Reverse Osmosis Treatment of Clean Condensate: Effects on Final Effluent Toxicity and Fish Endocrine Disruption. M.G. Dubé and D.L. MacLatchy. University of New Brunswick, Saint John, NB.

In March of 1998, Irving Pulp and Paper in Saint John, New Brunswick installed a Reverse Osmosis (R.O.) system to treat 1100 g/min of clean condensate leaving the fifth effect evaporator in the chemical recovery process. This installation followed a multi-million dollar Environmental Improvement Project to meet environmental effluent targets with "in-plant" technologies rather than end-of-pipe effluent treatment. The R.O. system has had a dramatic effect on final effluent quality resulting in approximately 17% and 12% reductions in biochemical oxygen demand and chemical oxygen demand, respectively. More importantly, the final effluent is non-acutely lethal to *Daphnia magna* (48 h LC_{50}) and rainbow trout (96 h LC_{50}) in the absence of secondary effluent treatment. Sublethal toxicity (IC_{25}s) of the final effluent to three marine test species is better or equal to levels reported by other Canadian mills with advanced effluent treatment. Mesocosm studies conducted before (August 1997) and after (July and August 1998) installation of the R.O. system showed that the threshold for endocrine disruption (plasma steroid hormone depression) in the mummichog (*Fundulus heteroclitus*) increased in 1998 compared to 1997. Effluent characterization studies and laboratory exposures are underway to confirm the significance of the R.O. process change on effluent quality.

Effects of 17β-Estradiol Exposure on Metallothionein and Fat Soluble Antioxidant Vitamins in Juvenile Lake Trout. V.P. Palace[1,2], K. Wautier[1], C.L. Baron[1], R.E. Evans[1] and J.F. Klaverkamp[1,2]. [1]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; [2]Department of Zoology, University of Manitoba, Winnipeg, MB.

Contaminants that mimic the effects of estrogen have been identified in Canadian waters. Previous work also shows that estrogenic contaminants can affect the metabolism of protective biochemical factors. The potential for estrogenic compounds to influence metallothionein (MTN) and fat soluble antioxidant vitamins (A and E) was investigated in lake trout (260±15 g) injected with ethanol/corn oil (control) or 5 mg/kg 17-β estradiol in ethanol/corn oil. Hepatic MTN was lower in estradiol treated fish versus controls. Lower copper and cadmium concentrations were also found in the liver of estradiol treated fish. Hepatic Zn concentrations were not different between the two groups. The estradiol group had higher concentrations of MTN in the kidney, but only copper was elevated in this tissue compared to the controls. Plasma vitamin A (retinol) and E (tocopherol) were elevated after 7 d in the estradiol group compared to the controls. Vitamin A returned to control values after 14 and 21 d in the estradiol group, but vitamin E remained elevated at 21 d post-exposure. Liver vitamin E and A were depleted in the estradiol group, but kidney vitamin levels were similar in both groups. Implications for increased susceptibility to the toxic effects of other contaminants, due to MTN and vitamin depletion in fish exposed to estrogen analogs, remains to be investigated.

Exposure to Waterborne Ethynylestradiol Alters Fat Soluble Vitamin and Lipid Metabolism in Juvenile Lake Sturgeon. V.P. Palace[1,2], T.A.Dick[2], K. Wautier[1], C.L. Baron[1], R.E. Evans[1] and J.F. Klaverkamp[1,2]. [1]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg; [2]Department of Zoology, University of Manitoba, Winnipeg, MB.

Studies in mammals have shown that exposure to estrogenic compounds can affect lipid metabolism and plasma concentrations of lipid soluble vitamins. However, the potential for estrogenic contaminants to induce these effects in fish has not yet been examined. The ability of the estrogen analog, ethynylestradiol (EE2), to alter concentrations of the lipid soluble vitamins A and E in plasma and in other tissues was investigated in lake sturgeon (430±20g). EE2 was delivered to the sturgeon in the tank water at concentrations of 0 (control), 15, 60 or 125 ng EE2/L for 25 d. Plasma vitamin E (tocopherol) and A (retinol) were elevated by EE2 treatment compared to the controls. Liver vitamin E and A were also elevated in the EE2 group, but kidney vitamin levels were similar in all groups. Total lipid content, estimated gravimetrically, was elevated in liver and gonad of the EE2 treated fish compared with the controls. Altered lipid and vitamin metabolism may be induced by estrogen to facilitate gonadal maturation in female fish. However, results from these experiments indicate that an examination of the implications for vitamin depletion by estrogenic contaminants in juvenile fish is warranted.

Effects of Water-borne 4-Nonylphenol and Estrogen on Atlantic Salmon (*Salmo salar*) **Smolts.** W.L. Fairchild[1], J.T. Arsenault[1], K. Haya[2], L. Burridge[2], J.G. Eales[3], J. Sherry[4], D. Bennie[4] and S.B. Brown[4]. [1]Fisheries and Oceans Canada, Gulf Fisheries Centre, Moncton, NB; [2]Fisheries and Oceans Canada, Biological Station, St. Andrews, NB; [3]Department of Zoology, University of Manitoba, Winnipeg, MB; [4]Environment Canada, National Water Research Institute, Burlington, ON.

A recent study found significant relationships between historical applications of an insecticide containing 4-nonylphenol (4-NP) and catch data for Atlantic salmon (*Salmo salar*) populations, suggesting possible declines in catch related to effects at the smolt stage. To test the hypothesis that 4-NP impairs parr-smolt transformation (PST) we exposed Atlantic salmon smolts to environmentally relevant, pulse doses of water-borne 4-NP. To determine whether 4-NP is operating via its properties as a weak estrogen, smolts were also exposed to sustained doses of estradiol (E2). The smolts capability to withstand sea water and their subsequent growth and survival were evaluated after exposure to nominal concentrations of 20 and 200 µg/L 4-NP, and to 100 and 300 ng/L E2. Osmoregulatory (plasma and tissue ions, gill ATPase), biochemical (glucose/glycogen), and endocrine (thyroid hormones, vitellogenin, cortisol) parameters were measured on smolts throughout the experiment. There were no treatment related increases in mortality during a sea water challenge soon after exposure. Subsequent growth and survival in sea water was impaired in about 25% of fish from the various treatment groups (5% in control). The response was bimodal, with the affected fish showing weight loss from soon after treatments. If the effects exerted by 4-NP are due to its estrogenic potential, then estrogenic activity stemming from other sources (e.g., domestic sewage, agricultural wastes or phytoestrogens from pulp mills) might influence present day salmon populations.

Vitellogenin Induction as a Biomarker of Exposure to Environmental Estrogens. K. Sobey[1], J.S. Goudey[2] and R. Robinson[3]. [1]Golder Associates Ltd., Saskatoon, SK; [2]HydroQual Laboratories Ltd., Calgary, AB; [3]Golder Associates Ltd., Calgary, AB.

A growing list of environmental contaminants have been reported to disrupt the vertebrate endocrine system. Alterations by environmental endocrine disrupting chemicals (EDCs) may affect hormone dependant processes such as growth and reproduction. Environmental estrogens (EEs) are the most widely studied of all the endocrine disruptors. EEs cannot be identified from structure alone because they come in differing shapes and sizes. This makes it difficult to predict which chemicals will produce estrogenic effects in living organisms. Identification is further complicated because of the number of diverse mechanisms in which a toxic effect may be elicited. Chemicals that have been reported to possess estrogenic properties include breakdown products of detergents, pesticides, plasticizers and a variety of chlorinated compounds. Vitellogenin (Vg) is a large lipoglycophosphoprotein which is normally synthesized by hepatocytes of female oviparous (egg laying) vertebrates. During the reproductive cycle of female salmonid fish, plasma concentrations of Vg have been reported to increase approximately one millionfold. In contrast, very little if any Vg can be detected in male fish, presumably because circulating estrogen concentrations are too low to trigger expression of the Vg gene. Although the Vg gene is normally silent, it can be induced if male fish are treated with estrogens. Exposure of male fish to various concentrations of both natural and synthetic estrogens has shown pronounced dose-response effects and has also shown that male fish are very sensitive to estrogens present in water. It is this large range of potential Vg concentrations that provides the ideal basis for a very sensitive bioassay of estrogen exposure in fish. Vg induction has been documented in a variety of fish species, as well as, amphibians, reptiles and birds. This makes testing for vitellogenin useful as a biomarker of estrogenic activity over a wide range of animals.

Defensible Endpoints for Studies on Endocrine Disrupting Chemicals. A.J. Niimi. Department of Fisheries and Oceans, Canada Centre for Inland Waters, Burlington, ON.

One of the more difficult aspects of endocrine disrupting chemical (EDC) studies is to establish linkages between chemical exposure and effects on organisms. Endocrine systems secrete chemical messengers that act through a series of negative feedback processes to maintain homeostasis in an organism. EDCs can act as an agonist or antagonist. Interpreting EDC attributed changes can be difficult because responses and effects are often graded and rarely amenable to yes-or-no observations. Changes in enzyme levels and their by-products are responses that may lead to an effect, but responses may not be the desired goal. Growth is an effect where increments are strongly influenced by external factors such as food availability and seasonality, demonstrating impairment in feral organisms can be a difficult task. EDC induced change to membrane permeability is another graded effect, although death is the endpoint often monitored. Structural changes reported in gonads at the cellular and organ levels can be considered an effect, but further studies are required to determine if these changes impair reproduction. Exposure to EDCs can determine the sex of an organism, but gender is not a deleterious effect, and a skewed sex ratio at the population level is a graded effect whose consequences are unknown. Public concerns have raised EDCs to a high priority issue, hence, it is imperative that we formulate working hypotheses and investigate goals to establish cause-and-effect relationships, and assess if these effects are deleterious, of no apparent consequences , or possibly advantageous to the organism.

A Quantitative Decision Framework for Evaluating the Value of Endocrine Disruption Test Information. R.C. Lee[1], L.W. Smith[2], J.S. Goudey[3] and S.M. Swanson[1]. [1]Golder Associates Ltd., Calgary, AB; [2]Research Institute for Fragrance Materials, Hackensack, NJ; [3]HydroQual Laboratories, Calgary, AB.

Regulatory agencies have designed testing protocols for determining the potential for endocrine disrupting chemicals (EDCs) to cause effects in aquatic systems. Many jurisdictions are following the recommendations of the US Environmental Protection Agency's Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC). This protocol is a tiered system for screening chemicals using a sequence of *in vitro* and *in vivo* tests, following general strategic principles of effectiveness and comprehensiveness. However, at present there is no formal analytic structure for relating the results of these tests to receiving environment effects at typical environmental doses, and no framework for evaluating the information value of these tests in terms of making optimal, cost-effective decisions regarding control of potential EDCs. The present effort proposes a quantitative, decision analytic framework for evaluating the uncertainty associated with the value of the EDSTAC-type test system in predicting environmental effects in aquatic organisms. Similar frameworks have been used for evaluating potential human carcinogens. This framework can use either statistical sensitivity, specificity, and predictive value when this information is available, or alternatively more powerful Bayesian influence diagram methods incorporating expert judgement. Economic costs of testing as well as costs to industry of controlling or banning potential EDCs can be explicitly incorporated into the analysis. Optimal decisions regarding the extent of testing necessary to determine the potential for endocrine disruption at environmental doses, as well as optimal cost-effective environmental control levels, are identified. Sensitivity of decisions to uncertainties in decision variables is estimated. Examples of the framework are presented.

Pulp and Paper Industry and Environmental Effects Monitoring

Fathead Minnow Long-term Growth/reproduction Tests to Assess Final Effluent from a Bleached Sulphite Mill. J. Parrott[1], C. Wood[2], P. Boutot[2], B. Blunt[1], M. 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 Operation, Edmundston, NB.

During the Cycle 1 Environmental Effects Monitoring (EEM) studies, yellow perch collected downstream of Nexfor Edmundston bleached sulphite mill had reduced gonad size and fecundity. On site 21 d exposures of goldfish to final effluent in 1997 confirmed the presence of steroid-disrupting compounds in effluent. After several mill process changes and improvements, final effluent from 1998 did not adversely affect goldfish steroids. To confirm improvements in final effluent, long-term fathead minnow (FHM, *Pimephales promelas*) growth and survival tests were carried out. A mobile flow-through bioassay trailer was constructed and set-up on site at the mill secondary treatment plant. Treatments included 1, 3.2, 10, 32, 49 and 100% final effluent with ethinyl estradiol (EE, 30 ng/L) as a positive control compound. FHM eggs were exposed <12 h after fertilization, and through to 30 and 90 d post-hatch. Survival was unaffected by exposure to BSM effluent, except for 100% effluent. FHM exposed for 30 d showed improved growth (weight, length) in BSM effluent (up to 49%), and decreased growth in 100%. To more realistically imitate environmental conditions, sand filtered and UV treated Saint John River water was used as the dilution water. This presented some larval mortality problems that were overcome with changes in length of time that larvae were contained in egg cups post-hatch. The ongoing research will demonstrate the feasibility and potential usefulness of on-site flow-through fish exposures. The work will continue over the next year, to assess the development, behaviour and reproduction of FHM. As well, Cycle 2 EEM will be conducted at this mill in the fall, and results from wild fish will be compared to lab exposures to determine the predictability of the long-term FHM exposures. The study is an example of industry-government-academia cooperative research project to determine methods for assessment of pulp mill final effluents under the EEM framework.

Seasonal Bile Metabolite Production and Mixed Function Oxidase (MFO) Activity: Correlation to Distance from Pulp Mill Effluent. D.E. Willis, J.D. Leonard and J. Hellou. Fisheries and Oceans Canada, Bedford Institute of Oceanography, Dartmouth, NS.

A variety of chemicals including tricyclic polynuclear aromatic hydrocarbons (PAHs) are known to be present in pulp mill effluents. HPLC analyses of gall bladder bile from Winter Flounder, *Pseudopleuronectes americanus*, trawled 1, 15 and 50 nautical miles from the effluent source, showed the presence of metabolites in a range indicative of di- to pentacyclic type PAHs. The spring or resting cycle samples had elevated concentrations of tricyclic type metabolites in bile of flounder collected near the effluent source, when compared to bile samples from the reference site. Although metabolites were observed in the summer or pre-spawning bile samples, the quantities detected were not correlated with sampling location or MFO enzyme activity. Mixed function oxidase activity (MFO), specifically 7-Ethoxyresorufin *O*-deethylase (EROD) was assayed in a sub-cellular fraction of whole liver homogenate from the fish used for the bile metabolite analyses. EROD activity from the spring survey was induced 3.5 fold in samples from the station nearest to the effluent source and decreased to 2 fold at the station 15 nautical miles from the source. No induction was observed in the summer samples obtained from these stations. Spring MFO measurements indicate an enhanced ability of fish to deal with foreign xenobiotics by metabolism possibly due to restricted movement vs seasonal migration. This capability degrades back to basal levels as spawning season approaches.

Use of Biotelemetry as a Tool for EAs and EEM Programs in the Mining and Pulp and Paper Industries – A Newfoundland Perspective. E.A. Luiker, B. Bennett and S. McKinley. Jacques Whitford Environment Ltd., St. John's, NF.

The use of biotelemetry as a tool for biological investigations has been increasing over the last 10 yrs due to recent advances in this technology. Initially, telemetry was developed to track the movements of fish or other animals in the field. By integrating physiological monitoring with telemetry, developments have been made to enable direct physiological measurements from fish or other animals in controlled situations (i.e., swim tanks) or in their natural environment. For example, biotelemetry tags have been developed to monitor EMG activity in fish muscle, heart rate and cardiac output, and swimming performance. These developments increase the ability to assess anthropogenic influences on biological receptors. Of particular interest to industry and regulatory agencies is the monitoring of physiological stress during exposures to industrial effluent. This information can then be used to determine concentrations of effluent that represent chronic effects threshold levels to fish. Biotelemetry has been employed in several projects in Newfoundland and Labrador, including Voisey's Bay environmental baseline characterization –

anadromous Arctic charr migrations in a sub-tundra region; Labrador Hydro Project environmental baseline characterization – fish migration and habitat use within the Lower Churchill River; and fish passage investigations; and the effectiveness of mitigations on the island of Newfoundland. The use of biotelemetry as a monitoring tool in offshore oil industry is being assessed.

Environmental Effects Monitoring (EEM) of Pulp and Paper Mills in Quebec: Summary of the Cycle 2 Results on Toxicity. I. Matteau, F. Perron and C. Langlois. Environment Canada, Environmental Protection Branch, Montreal, QC.

As part of the EEM done in accordance with the federal pulp and paper effluent regulations, 41 mills discharging their effluents in freshwater must twice a year (January-April and July-October) subject their effluent to three toxicity tests: growth of the green algae *Selenastrum capricornutum*, survival and reproduction of the water flea *Ceriodaphnia dubia*, and survival and growth of the fathead minnow *Pimephales promelas*. The results compiled in this study are based on three different samples per effluent and cover the period of summer 1997, winter 1998 and summer 1998, for a total of 137 analyzed samples. It must be noted that a large variability is present in the test results, between the mills on the one hand, and for the same effluent during the various sampling periods on the other. Results show that the lethal toxicity for the water flea and the minnow is almost non-existent, while most of the effluents display a sublethal toxicity, especially for the reproduction of the water flea. According to our data, the type of secondary treatment seems to have little effect on the measured toxicity, which clearly diminishes, however, in relation to the toxicity measured after only a primary treatment. As for the various processes used by the mills, the data does not single out a particular tendency with regards to the sublethal toxicity of their effluent. Results also show that, for many of the tests, there are certain connections between the SS and BOD contents of the effluent and its toxicity.

Environmental Effects Monitoring at 22 Ontario Pulp and Paper Mills Interim Report: Summary of EEM Effluent Toxicity Results for Four of Six Cycle 2 Sampling Periods. A.I. Borgmann, S. Humphrey and S. Michajluk. Environment Canada, Environmental Protection Branch, Downsview, ON.

Canadian pulp and paper mills are required to collect effluent samples on a semi-annual basis over a three-year period during Cycle 2 Environmental Effects Monitoring Program (Annex 1 to EEM/1997/1) and assess the samples for survival (acute toxicity), growth and/or reproduction (sublethal toxicity) effects in three test organisms, including larval fish, an invertebrate and an aquatic plant. From a partial Cycle 2 data set from data submitted by Ontario mills during four sampling periods, July 1 to October 31, 1997 and 1998, and January 1 to April 30, 1998 and 1999, there appears to be a substantial improvement in effluent quality since Cycle 1. Because secondary treatment has been installed at 75% of the mills in Ontario following Cycle 1, the installation of secondary treatment might be the reason for this improvement. In addition, there may be a connection between sublethal toxicity and the type of secondary treatment system (i.e., Aerated Stabilization Basins versus Activated Sludge Treatment Systems). Further investigation into the causes of sublethality could potentially identify the sources of the problem at each mill whose effluent is showing sublethal effects. However, because effluent quality is also affected by factors such as furnish composition or mill process changes, all factors must be considered before any definitive conclusions can be drawn.

Methodologies

Listening to the Animals: Controlled Field Experimentation to Emphasize Internal Organismal Exposure and Effects in Receiving Waters. S.M. Salazar and M.H. Salazar. Applied Biomonitoring, Kirkland, WA.

Standard protocols are being developed for field bioassays using caged bivalves. A draft standard guide was first submitted to ASTM in 1997 and is in the final stages of the review process. A standardized approach for collecting synoptic exposure and effects data will provide a better method for comparing results from different studies. These protocols also provide a means to move beyond chemical criteria for water and sediment toward developing criteria for tissue chemistry and bioeffects that account for the site-specific effects of receiving waters. Previous studies have shown that an exposure-dose-response triad approach can be used to reduce uncertainty in ecological risk assessments. Chemical exposure is characterized by measuring chemicals in water and sediment, the dose is characterized by measuring chemicals in tissues, and effects are characterized by quantifying an easily measured endpoint such as growth. Emphasis of the standard protocols has been placed on quantifying exposure and effects over space and time and combining the experimental control characteristic of laboratory bioassays with the environmental realism of traditional field monitoring. Recently, regulatory agencies have placed more emphasis on bioaccumulative substances of concern in the risk assessment process. The U.S. Environmental Protection Agency (USEPA) and the U.S. Army Corps of Engineers (USACOE) are developing tissue residue effects databases as a means of pairing exposure and effects data for predictive purposes. Examples will be provided to demonstrate approaches and applications of the caged bivalve methodology and the advantages of measuring exposure and effects under natural conditions in the receiving waters.

A Dialysis Mini-peeper for Measuring Pore Water Metal Concentrations in Laboratory Sediment Toxicity and Bioavailability Tests. L. Doig and K. Liber. Toxicology Centre, University of Saskatchewan, Saskatoon, SK.

We have modified a classical peeper (dialysis cell) design to create mini-peepers that are of convenient size for direct pore water sampling in laboratory sediment toxicity and bioavailability tests. A series of experiments placing peepers in Ni and Zn solutions, two size fractions of Ni-spiked sand, and two field-collected, Ni-spiked sediments, were conducted to evaluate peeper efficacy for measuring overlying water and pore water metal concentrations. Results showed that, in water-only experiments, the peeper samples were not significantly different from the surrounding solution by 48 and 96 h for Ni and Zn, respectively. Peeper chambers in the sand experiments equilibrated with the surrounding pore water in $\leq$120 h. In both trials with natural sediment, peeper equilibrium occurred in $\leq$48 h. Ni flux into the peeper cells during the first hour of experimentation showed that the rate of equilibration was initially related to media porosity. In fine sand and natural sediment equilibration was influenced not only by Ni diffusion into the peeper chambers, but also by a decrease in pore water Ni due to sediment sorption which accelerated peeper equilibration by reducing the difference between the peeper cell and the surrounding pore water solution. Overall, mini-peepers equilibrated with the surrounding pore water or overlying water within a time period sufficiently short to allow for the use of mini-peepers in routine sediment toxicity and metal bioavailablity experiments. Sampling precision using mini-peepers was comparable (CV=10.8%) to that obtained by conventional sediment centrifugation and filtration (CV=10.5%).

To Split or Not to Split: Impacts on Marine Benthic Invertebrate Numbers of Organisms and Taxa with "Quarter" and "Half" Laboratory Subsampling. L. Young and M. Davies. Hatfield Consultants Ltd., West Vancouver, BC.

Abstract
The Technical Guidance Document (TGD) for EEM Cycle Two for the pulp and paper industry states that subsampling of an invertebrate sample in the laboratory is acceptable, provided that the method used is quantitative. The TGD states that a minimum of one-quarter of the sample should be enumerated.

Hatfield Consultants Ltd. conducted two marine benthic invertebrate programs for Cycle Two in which at least one field subsample from each station was split (subsampled) in the laboratory into quarters. Each quarter was individually sorted, and organisms enumerated and identified. This paper compares the number of organisms and the number of taxa found in each quarter split, and relates these to the total number of organisms and taxa for the total field sample. In addition, quarter subsamples are combined to simulate "half" subsamples, and these data are also presented.

From these samples, the number of organisms was predicted relatively well at one mill, especially with samples which contained >100 organisms. Data indicate that samples with less than 100 organisms should not be split. The number of taxa, however, increased with increasing size of subsamples. Quarter samples contained on average 48% of the total sample's number of taxa; half samples contained 71% of the total taxa.

Methods for Field Samples
Benthic invertebrate samples were collected from both mills using a 12-in Smith-McIntyre dredge (area 0.087 m^2) from a platform of a commercial fishing vessel. Only grabs that were at least 3/4 full were kept for benthic invertebrate analyses. Samples were field-sieved immediately using a wash tub with 500 µm stainless steel mesh. Sieved samples were placed into hard plastic jars and preserved with buffered formalin. Samples were shipped to the consulting taxonomists, where sample splitting and further sieving were conducted.

Methods for Laboratory Subsampling
Mill A: The field sample was initially sieved at 1 mm to remove mud and formalin. A Folsom plankton splitter was used for splitting samples. The splitter was leveled so that it yielded exactly equal quantities, as measured with water and graduated cylinders. The sample was poured into the splitter; any residue in the sample jar was washed with a squirt bottle. The splitter was agitated until the solids appeared to be evenly distributed in the splitter, and then the material was poured off into the two receiving boats. This procedure was repeated with these subsamples to produce quarter samples. Each quarter fraction was then put into a sorting dish and processed under the dissecting microscope.

Mill B: The field sample was initially sieved at 3 mm and 1 mm to remove the coarse fraction (>3 mm). The fine material (1-3 mm) was placed into a flat-bottomed pan and gently shaken or agitated so that sample materials were evenly distributed over the pan bottom. If any clumping of material was observed, the clump was carefully "teased" apart for even distribution. A grid with four (4) sections was placed into the pan and carefully worked through the sample material until the rubber on the bottom of the grid contacted the bottom of the pan. The grid was weighted down to secure a water-tight seal between sections. Each quarter of the sample was then siphoned into separate containers and appropriately labelled. Large samples were processed in batches, with each subsample split to the level required. In addition, observations were made before the sample was processed to detect layering or clumping of materials prior to sample splitting.

Graphical Data/Statistical Analyses
For both sets of quarter and half splits, graphs illustrate numbers of organisms for each subsample, total numbers per sample, and the standard error of the mean (percent one standard deviation divided by the mean) (Figs. 1 to 4). This is compared to the Index of Precision discussed in the TGD for determining the number of field subsamples to collect - that precision is recommended at 20% (i.e., the standard error is equal to 20% of the mean). Numbers of taxa found in each subsample are compared with the total for each sample given as a percentage of the total number of taxa (Figs. 1 to 4). Given the required level of identification to species or genus for most organisms, taxonomic lists are long with few individuals found per taxa in each sample. Analyses of overall numbers of taxa per station at each mill are included for discussion of "rare" taxa and the loss of some of these through laboratory subsampling.

Discussion
Abundance
Marine benthic invertebrate samples collected during Cycle 1 for these mills were sieved and identified at 180 μm size, which required subsampling in order to process the high numbers of juveniles and nematodes comprising the samples. For Cycle 2, the sieve size changed to 1 mm, with guidance to split at most to quarter subsamples. The TGD recommended that a sample or subsample contain a minimum of 100 animals for accurate characterization of the benthic community based on work completed by various investigators (e.g., Hickley, 1975; Elliott, 1977, cited in Environment Canada 1998). In our study, quarter subsamples of samples with 400 or more organisms (approximately 100 organisms per subsample) did not predict abundance more accurately than samples with fewer organisms (Fig. 1 and 3). Standard errors of the mean for quarter subsamples (>100 organisms) ranged from 20.7-28.1%, which is greater than the precision index suggested for field subsampling (20%). Quarter subsamples with 100 to 400 organisms in the total sample (25-100 organisms per subsample) exhibited standard errors ranging from 6.3-39.3%.

"Half" subsamples exhibited slightly greater precision, especially for Mill A subsamples (Fig. 2 and 4). Subsamples which comprised >100 organisms exhibited standard errors ranging from 1.8-15.2% (Mill A) and 6.0-32.8% (Mill B). Subsamples with <100 organisms exhibited standard errors ranging from 5.4-57.6%; the highest standard errors were often associated with samples which contained <100 organisms in total.

Conclusions
Splitting method may have had an effect on the accuracy of abundance estimates from subsamples based on these datasets. Subsamples split with the Folsom splitter yielded smaller standard errors of mean abundance relative to the tray-division method. Subsamples with >100 organisms did not necessarily provide more accurate estimates of abundance from subsamples;

however, samples with less than 100 total organisms exhibited large variability when divided into quarter subsamples. Based on these data, we recommend that samples with fewer than 100 organisms should not be split, and that any subsample should contain >100 organisms. Generally, it is best not to split a sample unless the analysis of the total sample will significantly increase costs.

Number of Taxa

The number of taxa found in these benthic invertebrate surveys was large (173 organisms at Mill A and 293 organisms at Mill B, adults and juveniles) given the requirement to identify polychaetes (in particular) to species. Polychaetes constitute the majority of organisms in marine benthic communities, and, consequently, a large number of individual species. Most taxa are represented by very few organisms, and common taxa represent only a small portion of the community (4-11% of abundance provided by 4 or 6 taxa). Overall relative abundance of taxa by rank indicates a large tail of "rare" taxa. When contemplating splitting samples, the probability that each taxa is represented in a quarter subsample, for example, is limited by its abundance (must be ≥4 organisms in total sample). Even when three samples are composited for one station, the number of taxa with at least 4 organisms ranged from 4-50% of the entire list at various stations. Therefore, at this level of taxonomic identification, splitting of samples will result in many missed "rare" taxa. Numbers of taxa increased with increasing numbers of organisms per sample or subsample (Figs. 1 to 4). Quarter subsamples comprised 29-61% of taxa found in the total sample; "half" subsamples comprised 57 to 79% of total taxa.

Questions arise as to the value of identifying benthic invertebrates to species if life history and tolerance to various pollutants is not known to that level of precision. The TGD discusses levels of impacts based on benthic communities and sediment chemistry impacts in terms 50% differences in abundance and number of taxa between exposed and reference areas. It may be sufficiently useful to identify invertebrates to family or genus to evaluate impacts at these large difference levels.

If information is available for life history and tolerance levels of species of marine organisms, identification to that level may be useful. However, the resulting taxa list contains many taxa represented by very few organisms. When samples are split, many of these "rare" taxa may be missed when only one subsample is analyzed. Based on these data, we suggest that samples not be split unless numbers of organisms are sufficiently great to significantly increase the cost of analysis. We also recommend that the level of taxonomic identification be reviewed.

References

Environment Canada. 1998. Pulp and Paper Technical Guidance for Aquatic Environmental Effects Monitoring. EEM/1998/1. April 1998.

Acknowledgements

Hatfield Consultants Ltd. would like to acknowledge the taxonomists who analyzed the samples and provided the raw data: Dr. Charles Low, Invertebrate Taxonomist, Victoria, BC, and Ms. Val Macdonald, Biologica Environmental Services, Victoria, BC. For more information, please contact the authors.

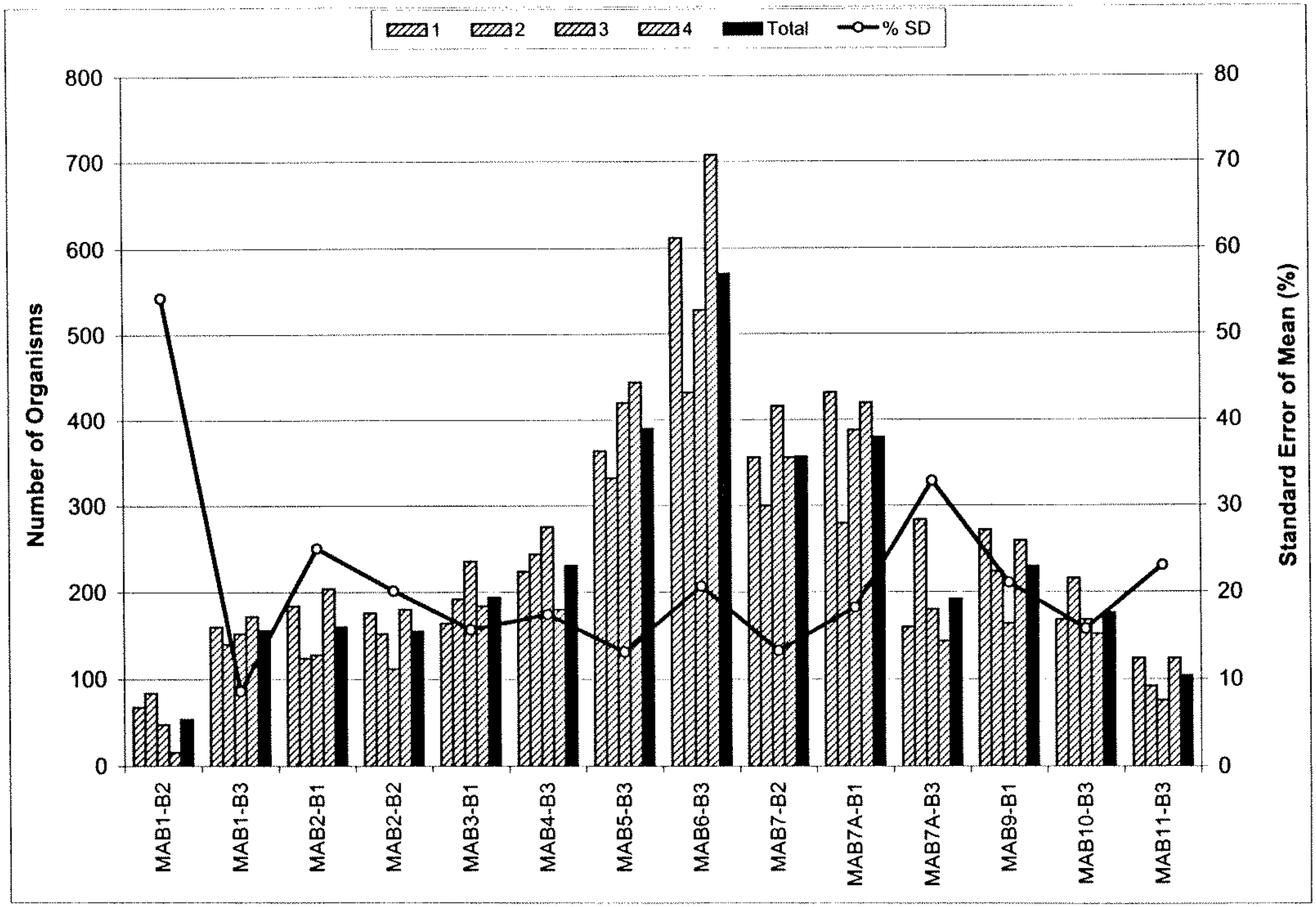


Fig. 1. Results of quarter subsamples of marine benthic invertebrate samples, Mill A. Numbers of organisms estimated from each quarter subsample and total number of organisms; standard error of the mean quarter subsamples (percent SD/mean per sample). Number of taxa for each quarter subsample and number of taxa found in total sample; percentage of average number of taxa for quarter subsamples relative to total taxa per sample.

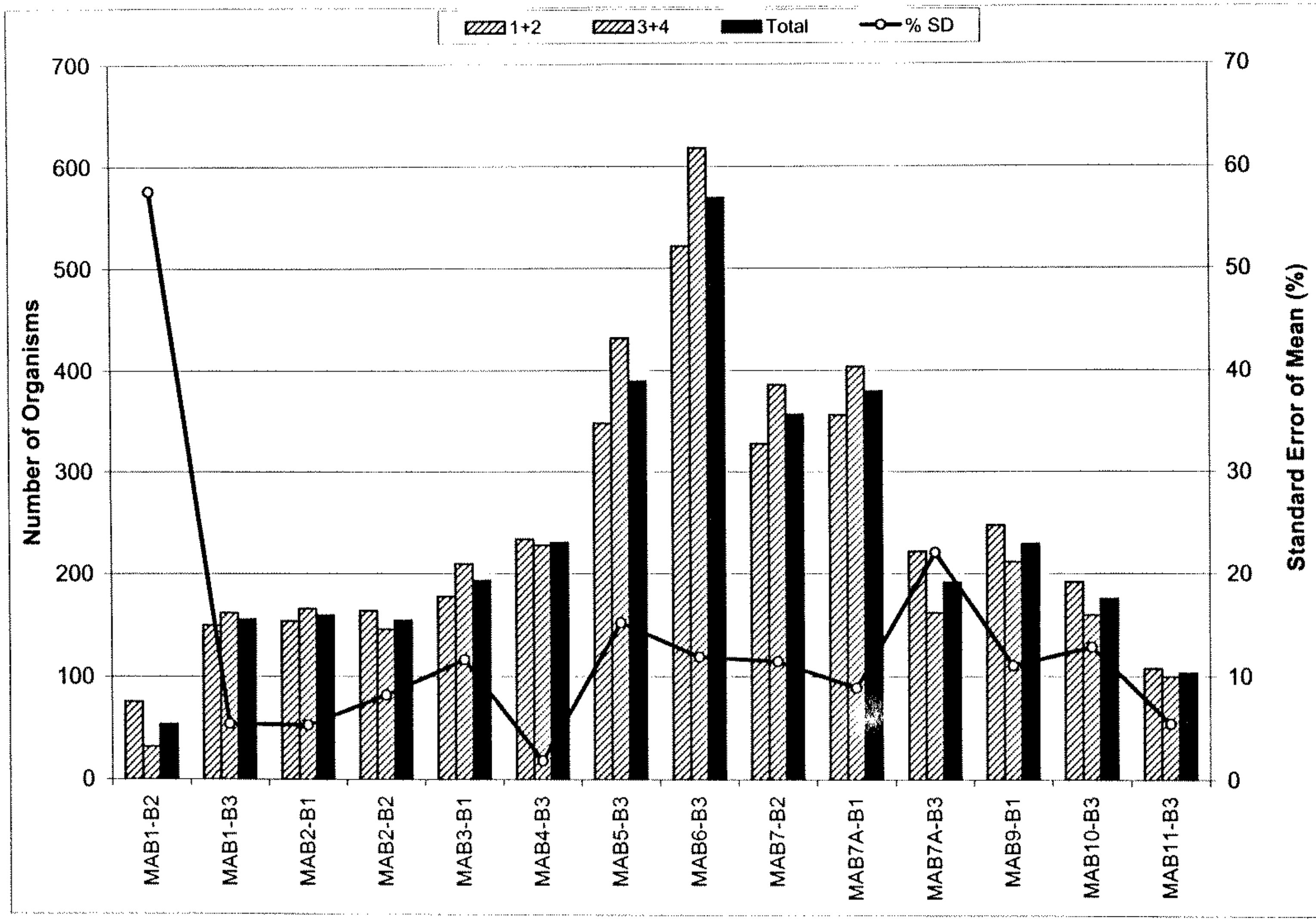


Fig. 2. Results of "half" subsamples of marine benthic invertebrate samples, Mill A. Numbers of organisms estimated from each "half" subsample and total number of organisms; standard error of the mean "half" subsamples (percent SD/mean per sample). Number of taxa for each "half" subsample and number of taxa found in total sample; percentage of average number of taxa for "half" subsamples relative to total taxa per sample.

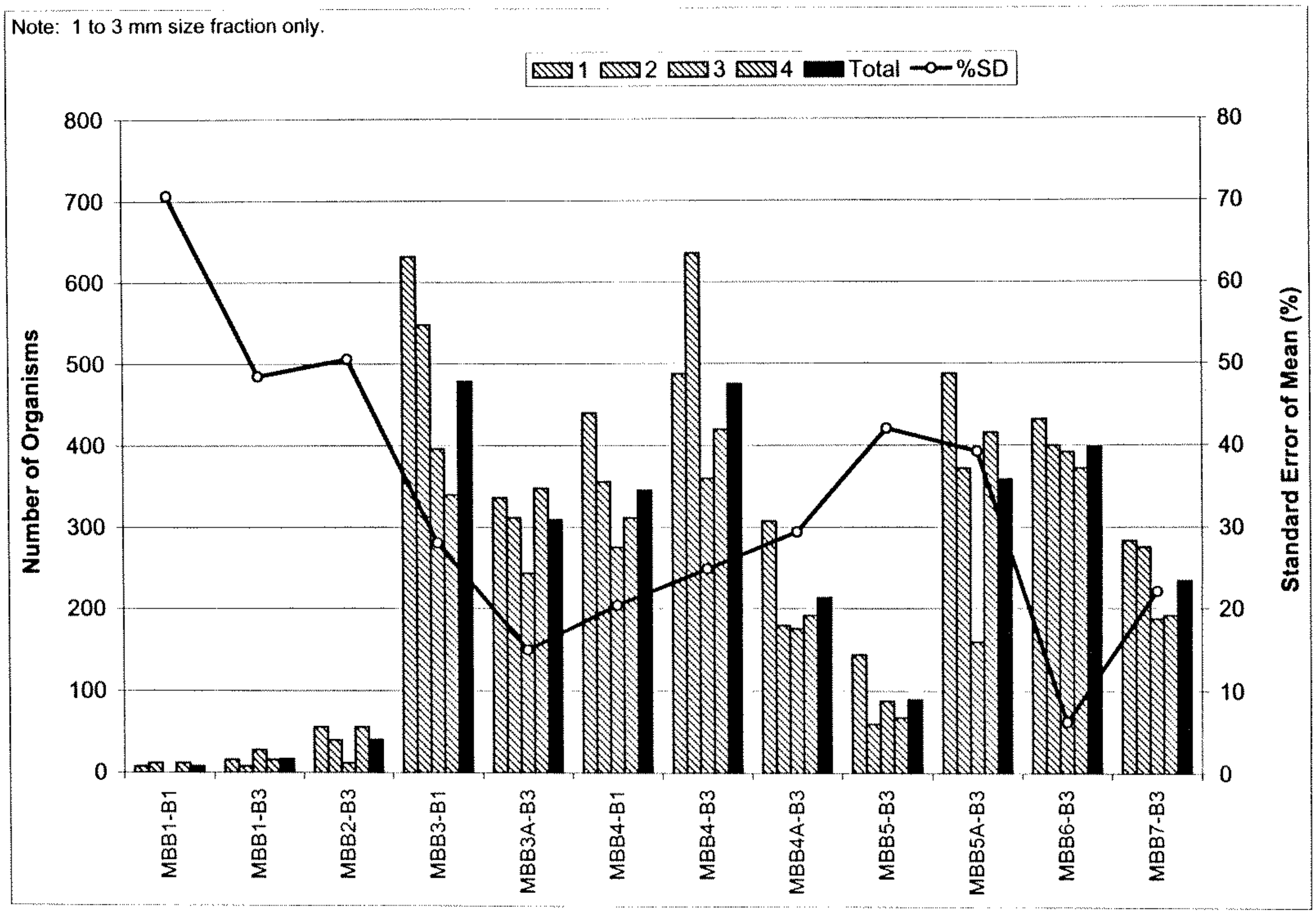


Fig. 3. Results of quarter subsamples of marine benthic invertebrate samples, Mill B. Numbers of organisms estimated from each quarter subsample and total number of organisms; standard error of the mean quarter subsamples (percent SD/mean per sample). Number of taxa for each quarter subsample and number of taxa found in total sample; percentage of average number of taxa for quarter subsamples relative to total taxa per sample.

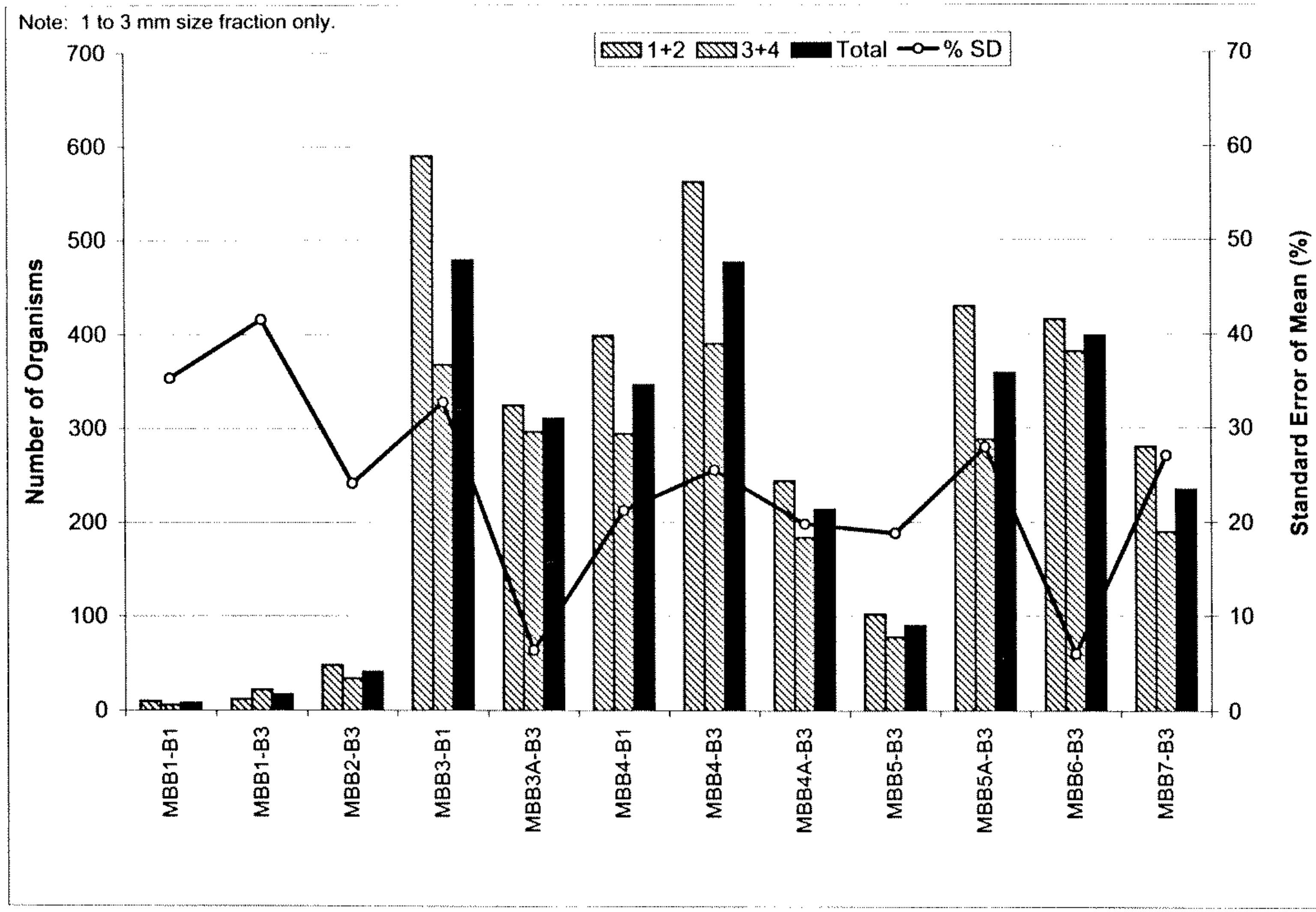


Fig. 4. Results of "half" subsamples of marine benthic invertebrate samples, Mill B. Numbers of organisms estimated from each "half" subsample and total number of organisms; standard error of the mean "half" subsamples (percent SD/mean per sample). Number of taxa for each "half" subsample and number of taxa found in total sample; percentage of average number of taxa for "half" subsamples relative to total taxa per sample.

Development and Application of Truss Analysis for the Determination of Fish Condition.

J. Nanson[1], D. Fitzgerald[1], T.N. Todd[2] and B.M. Davis[2]. [1]Department of Biological Sciences, University of Windsor, Windsor, ON; [2]USGS, Great Lakes Science Center, Ann Arbor, MI.

Conservation of skeletal structure and unique body ratios in fishes facilitated the development of truss analysis as a taxonomic tool to separate physically similar species of fish. The methodology involves determination of unique landmark sites and comparison of across-body measurements from a sequential series of polygons (trusses) with all landmark points within each polygon connected. Measurements that include substantial muscle composition and little skeletal support are not useful in taxonomic studies because they fluctuate with ration level; these measurements are relevant, however, for identifying differences in condition among individuals of the same species. We completed a controlled-feeding experiment using age-1 yellow perch (*Perca flavescens* Mitchill) with half the fish receiving a high ration (3.5% body wt/d) and the other half receiving a low ration (0.5% body wt/d). At 1, 2, 4, and 8 wk, both replicate ration groups of fish were removed from the study and photographed. Standard measures of condition (weight-length ratios, dry weight, total lipids) were compared to measurements determined from computer-digitized pictures of the fish. Principal components analysis identified truss measures from the caudal region of the yellow perch as important characters in changing fish condition. Potential exists to apply similar analyses on other fish species and use this approach for comparisons among habitats and even across geographic regions.

Utilization of Inductively Coupled Plasma (ICP) Spectroscopy to Determine Levels Of Heavy Metals in Black Rock Locks Sediments.

R. Culp, J. Shea, G. Handzlik, P. Schaber and P.F. Dehn. Departments of Biology and Chemistry, Canisius College, Buffalo, NY.

The EPA lists cadmium, lead and mercury as priority pollutants, while the IJC lists mercury and lead as critical pollutants for the Great Lakes. The purpose of this study was to utilize ICP spectroscopy to determine levels of these metals in surface sediments and to provide sediment extracts for bioassay work. Sediments were collected from the Black Rock Locks at the mouth of the Niagara River and extracted using EPA method 200.7. The extracts were split for quantification via ICP spectroscopy or for use in a bioassay. Minimal detection limits were 0.36, 43.81, and 5.18 μg/kg, while percent recoveries were 77.9%, 81.2% and 80.1% for Cd, Pb and Hg, respectively. Black Rock Locks sediments contained 16.7, 1034.6, and 2.2 mg/kg dry weight Cd, Pb and Hg, respectively. These are above the ranges found in the Times Beach Confined Disposal Facility, which is upstream from the BRL, for Cd and lead (1.4-5.9 and 195-637 mg/kg, respectively), but below levels reported for Hg (3.7-16.0 mg/kg). (Undergraduate researchers R.C., J.S. and G.H. were supported by a Merck/AAAS Interdisciplinary Research grant to Canisius College.)

Determination of Organochlorine Pesticide and Polychlorinated Biphenyl Concentrations in Black Rock Lock Sediments.

S. Soehnlein, M. Bradley, J. Crawford, G. Herman, R. Grebenok and P.F. Dehn. Departments of Biology and Chemistry, Canisius College, Buffalo, NY.

The EPA and the IJC lists several organochlorine pesticides and the polychlorinated biphenyls as priority or critical pollutants for the Great Lakes. The purpose of this project was to quantify levels of organochlorine pesticides and polychlorinated biphenyls in sediments from Black Rock

Locks. Surface sediments were collected from the Black Rock Locks at the mouth of the Niagara River. Sediments were extracted according to EPA method 3540c using a methylene chloride-hexane solvent system. Clean-up and fractionation was performed according to EPA method 36.20 on a florisil column. Three fractions were generated containing non-polar, partially polar, and polar molecules, respectively. The crude extract and each of the fractions were split for quantification via GC with electron capture detection or for use in a bioassay. Fraction 1 contained DDE, Mirex, PCB's and large concentrations of aromatics-most likely pthalates, Fraction 2 had plasticizers, but no measurable levels of p'p-DDT, nonachlor, or chlordane, Fraction 3 had a complex mixture of polar molecules as of yet unidentified. (Undergraduate researchers S.S., M.B., and J.C. were supported by a Merck/AAAS Interdisciplinary Research Grant to Canisius College.)

Utilization Of A Human Cellular Bioassay To Assess Microcystin-LR Toxicity. A.M. Carr and P.F. Dehn. Biology Department, Canisius College, Buffalo, NY.

Contamination of water by cyanobacteria (blue-green algae) has been found to cause serious public health problems. Microcystin, a potent hepatotoxin, is the most commonly known cyanobacteria and it is found in water supplies around the world. In the present study the effects of purified microcystin-LR was evaluated using a human carcinogenic liver cancer cell line (HepG2), which retains major cellular detoxification systems. Cells were exposed to a range of microcystin between 0.05 μM–50 μM for 24 or 72 h. After which, cell viability was tested using the neutral red assay, membrane damage was measured by lactate dehydrogenase (LDH) leakage, and detoxification ability was determined by measuring levels of glutathione (GSH). Previous studies report that *in-vivo* exposures to microcystin in rats and mice caused rapid tumor promotion and death to the liver cells. However, in this study no toxic response was observed in the HepG2 cells at concentrations lower than 50 μM or before 72 h of exposure. Viability and GSH levels decreased significantly at this concentration; however, membrane permeability was unaffected. Although the mechanism responsible for the cytotoxicity of microcystin has not been fully elucidated, the results from this study and other *in vitro* studies suggest that the toxin must be bioactivated to induce a toxic response, and that direct damage to the membrane is not responsible for toxicity. (Undergraduate researcher A.C. supported by a CEEP grant from Canisius College.)

Effect of Microtox Reagent Reconstitution Age on the Variability of Analytical Results from the Microtox® Assay. I.D. Gaudet and R.N. Coleman. Alberta Research Council, Vegreville, AB.

Please refer to paper on pages 59 to 70.

Microplate Assays with Unicellular Algae: Effects of Centrifugation and Cell Washing on Resolution of Endpoints. C.D. Christie, J.S. Hamilton and J.S. Goudey. HydroQual Laboratories Ltd., Calgary, AB.

Centrifugation and cell washing (replacement of the test solution with clear solution) are steps required in the Environment Canada algal growth inhibition test when endpoints are determined from optical density measurements (plate reader). The influence of these treatments on derivation of endpoints was examined in six different effluents and waters (no colour, coloured, and turbid after filtering). The IC_{25} and IC_{50} were derived and compared based on the following: actual cell counts; blank correction with a microplate containing test solution but no algae (t=0 and t=72 h); before and after washing and centrifugation; multiple washing and centrifugation (endpoints derived after each of three rinses); and measurement of optical density at multiple wavelengths. Centrifugation and replacement of the test solution had no significant effect on derived endpoints in optically clear solutions at non-absorbing wavelengths. The effects of turbidity (filterable) and colour could be accounted for with an appropriate blank (plate with diluted test solution and no algae). Suggestions are made for ways to determine if a test solution will confound resolution of accurate endpoints in 96-well microplate assay format based on optical density measurements; and how to make appropriate corrections.

Toxicity Testing with Volatile Substances. G. Stephenson[1], N. Koper[2], J. Princz[1] and P. Miasek[3]. [1]University of Guelph, Guelph, ON; [2]University of Alberta, Edmonton, AB; [3]Imperial Oil, Toronto, ON.

Current test methods for assessing soil toxicity required procedural modification during test soil preparation when spiking with highly volatile substances. A series of tests were initiated in an artificial (AS) and a field-collected reference soil (Ontario sandy-loam) to investigate alternative test preparation methodologies designed to minimize the volatilization of motor gasoline (mogas). Mogas contains predominantly low-end carbon components (C_5–C_{13}) with a volatility equal to about 1000 times that of napthalene. As losses can occur at different stages of test soil preparation, analyses of TPH extractables and BTEX/TPH purgeables allowed the loss at each stage to be quantified. Spiking at much higher concentrations than required for an adverse effect compensated for the rate of loss due to volatilization. Additional modifications compared mixing test soil by rolling it in an enclosed chamber versus traditional mechanical mixing. Spiking methodology contrasted spiking a batch of soil to "100% contamination" and diluting with clean soil versus spiking specific levels of mogas into soils to achieve specific exposure concentrations. Traditional open (for exchange of air) versus modified closed test units were compared in assessing organism survival and mogas toxicity. Acute and chronic toxicity tests employed the methodology that minimized the loss of the volatile components in mogas. The test methods developed to assess the toxicity of mogas in soil to a battery of test organisms may be equally applicable to the toxicity testing of other highly volatile substances.

Quality Control in Toxicity Testing - Control Charts and Criteria for Accepting Reference Toxicant Test Results. M. Stefanik and J.E. Schroeder. Beak International Inc., Brampton, ON.

Reference toxicant tests are integral to a laboratory quality control program. How their results are assessed is important in assuring the quality of all toxicity data for a particular test system and species. In Canada, results from reference toxicant tests are evaluated through the use of control charts, according to guidance provided in Environment Canada's guidance document for reference toxicants. However, limited guidance is available for determining appropriate action in a number of circumstances, such as when results fall outside the control limits; greater than 5% of results fall outside the warning limits without an associated cause; and trends in sensitivity are recognized within a control chart. Other issues that warrant additional discussion include the maximum variability (coefficient of variation) that is acceptable for specific tests and the relevance of logging concentrations when establishing a control chart. For example, when the geometric mean and standard deviation are used to prepare a control chart, control and warning limits are shifted as compared to using the arithmetic mean and standard deviation. This presentation will review current Canadian, U.S. and international guidance and will present recommendations for approaching the above issues. In addition, relevant outcomes from the Environment Canada Statistics Workshop, scheduled for September, 1999, will be provided.

Chemical Fate and Toxicity

The Effect of Ultraviolet Light on the Toxicity of Sediments Collected from Kitimat and Kildala Arm of Douglas Channel, Kitimat, BC. C. Eickhoff[1], J. Pickard[1], K. Omotani[2], K. Kinnee[1] and S. Lis[1]. [1]BC Research, Inc., Vancouver, BC; [2]Alcan Primary Metals Group, Kitimat, BC.

The objective of this study was to assess the potential acute and sublethal phototoxic effects of PAH contaminated sediments on marine aquatic organisms in the water column. The sediments were collected from two sites in Kitimat arm, D Lagoon Foreshore and Inner Harbour Intertidal, and from a reference site in Kildala Arm. Both Kitimat and Kildala Arm are at the head of Douglas Channel on the coast of northern British Columbia. Sediment elutriates were prepared by rotating the sediments with seawater for 30 min, centrifuging for 15 min and decanting the elutriate. Three marine species were used to assess phototoxicity of the elutriate: larval topsmelt (*Atherinops affinis*), juvenile mysid shrimp (*Mysidopsis bahia*), and embryonic mussel (*Mytilus galloprovincialis*). Survival (all species), growth (topsmelt) and embryo development (*Mytilus*) were measured. Each test was performed with both UV light treatment and normal laboratory lighting. There were no acute lethal effects due to UV light treatment or sediment elutriate type observed in any of the tests. The UV light treatment appeared to have a direct negative effect on sublethal endpoints in controls such as topsmelt growth and mussel embryo development, however, PAH associated phototoxic effects were not observed. Although there were detectable levels of PAH present in the sediment elutriates of Kitimat Arm, no negative sublethal effects linked to sediment elutriate concentrations were observed. The PAH determined in the sediment elutriates may have been insufficient in concentration or may have interacted in some manner with suspended particles and were non-photoreactive.

The Effects of Processed Kimberlite Ore Effluent on *Ceriodaphnia dubia*. 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.

In the fall of 1998, BHP's Ekati Diamond Mine began operations in Canada's Northwest Territories. In its operation, the diamonds are mechanically extracted from the crushed ore (kimberlite) and the fine tailings are deposited into the Long Lake Containment Area. While the diamond extraction process is chemical free, coagulants are added to the ore fines (≤0.5 mm size) to facilitate settling in the containment area. At the present time all of the effluent is contained within the tailings containment area, but the liquid will eventually be discharged into the Lac de Gras drainage basin. Very little is known about the effect of processed kimberlite ore effluent (PKOE) on aquatic organisms. In order to evaluate the toxicity of PKOE to freshwater zooplankton populations, a series laboratory evaluations were conducted using *Ceriodaphnia dubia* as the test organism. Acute (48 h) and chronic (7 d) toxicity tests were conducted with supernatant and filtrate (0.45 μm) of the final process effluent. Neither the supernatant nor the filtrate, at 100% strength, were acutely toxic to *C. dubia*. Chronic toxicity studies revealed that growth, reproduction and eventually survival were adversely affected by undiluted effluent supernatant and filtrate. A serial dilution chronic study indicated that effects on survival occurred at an effluent filtrate concentration of approximately 50% and that effects to reproduction occurred at effluent filtrate concentrations lower than 25%. Results from a series of effluent Toxicity Identification Evaluations, using *C. dubia*, will also be discussed.

Toxicity of Uranium, Nickel and Arsenic to *Hyalella azteca* in Spiked-sediment Toxicity Tests. K. Liber and S. Sobey. Toxicology Centre, University of Saskatchewan, Saskatoon, SK.

Northern Saskatchewan is home to several active uranium mines. Effluent from these mines generally contains elevated concentrations of several metals and metalloids resulting in localized increases in toxic metal/metalloid concentrations in aquatic receiving environments. At the Rabbit Lake, SK, uranium mine the primary metals/metalloids of concern include U, Ni and As. A series of laboratory spiked-sediment toxicity tests with the amphipod *Hyalella azteca* were undertaken to determine acute and chronic toxicity thresholds for U, Ni and As based on both total sediment and pore water concentrations. Water-only toxicity data were also generated for the three elements for comparison to pore water metal concentrations in the spiked-sediment toxicity tests, and to evaluate the hypothesis that pore water metal concentrations are more strongly correlated with sediment toxicity to benthic organisms than are total metal concentrations. Based on measured, whole sediment toxicant concentrations, Ni and As were the two most toxic elements tested, followed by U (LC_{50}s for *H. azteca* were 521, 532, and 2,442 mg/kg d.w., respectively). IC_{50}s based on growth were marginally lower than LC_{50}s. Based on measured pore water concentrations, U and Ni were the most toxic elements with LC_{50}s for *H. azteca* of 2.15 and 2.05 mg/L, respectively. As followed with an LC_{50} of 27.75 mg/L. Growth was always a more sensitive endpoint than survival. The lowest IC_{50} was observed for Ni (0.92 mg/L), closely followed by U (2.27 mg/L). The significance of pore water vs. total metal concentrations for interpreting sediment toxicity will be discussed.

Metal Mixture Toxicity in Aquatic Invertebrates. C.P. Cabral[1], U. Borgmann[2] and G. Dixon[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Environment Canada, National Water Research Institute, Burlington, ON.

Despite the fact that metal contamination of the aquatic environment is rarely the result of a single metal, all regulatory standards for metals in North America are based on single metals. Although numerous studies on the toxicity of metals in mixtures have been conducted, no clear and consistent pattern has emerged. An extensive examination of the literature, points to many different directions. Any given combination of metals (e.g., Cd+Zn) can result in additive or antagonistic responses when toxicity is expressed in terms of concentrations of metals in water. Body concentrations are often a more useful indicator of toxic effects than water concentrations in studies with single toxicants. Thus, it is likely that they will also be better indicators of toxicity in metal mixtures. In this study, we analyzed four major industrial contaminants, i.e., Cd, Cu, Pb and Ni. LC_{50} values were obtained for each metal singly and in a mixture. The experiments were carried out in 500 mL Erlenmeyer flasks, in a 250 mL five-salt artificial medium (SAM-5S) using the Amphipod *Hyalella azteca* and lasted one week. The animals were cultured in the Laboratory at Canada Centre for Inland Waters in Burlington where the experiments were also conducted. At the end, the remaining living animals were counted, placed in 1 mL cryovials, and put in a 60 °C oven to dry. The tissues were digested in nitric acid and analyzed for metal contents in an Atomic Absorption Spectrophotometer. The Toxic Unit (TU) model was used, giving a value of 2.3 TU's, which suggests an independent mode of action for the mixture.

The Effects of Azamethiphos on Survival and Spawning Success in Female American Lobsters (*Homarus americanus*). L. Burridge, K. Haya and S.L. Waddy. Fisheries and Oceans Canada, Biological Station, St. Andrews, NB.

In southern New Brunswick, Canada the period of peak sea lice infestation and treatment is the summer and autumn. This time is coincident with spawning of the American lobster (*Homarus americanus*). Salmonid aquaculture sites and lobster nursery areas share the same water, resulting in a situation in which lobsters may be exposed to effluent from sea lice treatments. The only pesticide registered for use against sea lice in Canada is the organophosphate azamethiphos, an acetylcholinesterase inhibitor. Preovigerous female American lobsters (n=72) were divided into three groups (two treatment and a control) and ovarian maturation and spawning was induced using elevated water temperature and short day length. Lobsters were exposed four times for one hour to either 10 or 5 µg/L of azamethiphos. These concentrations represent 10 and 5% of the recommended treatment concentration. Treatments were separated by two weeks. Survival and success of spawning were monitored. After the fourth exposure to 10 µg/L azamethiphos 43% (10) of the lobsters had died, only one was dead after the third treatment. In contrast, only 8% (2) of those exposed to 5 µg/L died (only after the final treatment) and there were no deaths amongst the controls. In a separate experiment the activity of acetylcholinesterase (AChE) in the muscle of exposed lobsters was measured. These data suggest a possible cumulative inhibition of this enzyme. Alternatively, increased sensitivity of the lobsters during the fourth treatment compared to earlier treatments may be related to seasonal differences in physiology or to the endocrine state of preovigerous and spawning females. Spawning success, as assessed by the presence of extruded eggs, was also affected by exposure to the highest concentration of azamethiphos. Eight (57%) of the surviving lobsters exposed to 10 µg/L failed to spawn, while 2 (9%) of the surviving lobsters exposed to 5 µg/L of azamethiphos and only 1 (5%) of the control lobsters failed to spawn.

Recent Additions to the Copper-gill Binding Model. M.L. Schwartz, N. Rose-Janes and R.C. Playle. Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Two new variables are being added to the existing mathematical model for copper binding to fish gills. These variables are Mg and Na, which should extend the use of the model from freshwater into brackish water and perhaps to seawater conditions. Mg at 2.2 mM (as $MgCl_2$) kept Cu off the gills of rainbow trout (*Oncorhynchus mykiss*, ~1 g, 3 h exposures, 0.4 µM Cu added as $CuSO_4$). Modelling using the $MINEQL^+$ aquatic chemistry program suggests the conditional equilibrium stability constant of Mg binding to trout gills is approximately ten times greater than that of Ca (log $K_{Ca\text{-}gillCu}$=3.4), about log $K_{Mg\text{-}gillCu}$=4.4. Na at a nominal concentration of 0.5 mM (as

NaCl) kept Cu off the gills of rainbow trout (~2 g, 3 h exposures, 0.4 μM Cu as $CuSO_4$), yielding a log $K_{Na\text{-}gillCu}$~4.3. The physiological effect of Cu to fish is a disturbance in Na uptake, so the higher log K value for Na than Ca appears reasonable. The greater protective effect of Mg compared to Ca is unexpected, and will require more work to determine if this result translates into a greater protective effect of Mg than Ca against Cu toxicity.

Well-being of Mussels (*Mytilus edulis*) **Collected from Halifax Harbour, as Reflected by Condition Indices, Lipid Content and the Presence of PAHs.** J. Hellou[1], T. King[1], B. Petrie[1], D.E. Willis[1], P. Yeats[1] and V. Zitko[2]. [1]Fisheries and Oceans Canada, Bedford Institute of Oceanography, Dartmouth, NS; [2]Fisheries and Oceans Canada, Biological Station, St. Andrews, NB.

Length, weight, moisture, lipid content and concentrations of parental and alkylated PAHs were determined in the filter feeders, *Mytilus edulis*. Animals were collected over three seasons at 17 sites covering nearly 30 km of shore line, including the island situated at the entrance to Halifax Harbour and a narrow tributary, the Northwest Arm. Condition indices were calculated (dry weight/length^3) and compared to lipid content and concentrations of PAHs to determine the health of the animals in space and time. Lipid content was generally highest in July and lowest in April, while condition indices tended to show an opposite variation with season. PAH concentrations were nearly undetectable in mussels collected in August, and highest in some pre-spawning mussels collected in April. At all sampling sites and seasons, fluoranthene was the predominant PAH of the suite of 30 analytes. The PAH profiles reflected proximity to combustion and petroleum sources and possible weathering. Results will be discussed and interpreted in terms of the water circulation in the harbour and various observations regarding this multi-species habitat that received untreated sewage effluents for the past 250 years.

Low Molecular Weight Non-*Ortho* Chlorobiphenyls in Mussels, *Mytilus edulis*, Collected in Halifax Harbour, Nova Scotia, Canada. T. King[1], D.E. Willis[1], V. Zitko[2] and J. Hellou[1]. [1]Fisheries and Oceans Canada, Bedford Institute of Oceanography, Dartmouth, NS; [2]Fisheries and Oceans Canada, Biological Station, St. Andrews, NB.

Chlorinated biphenyls (CBs) were measured in mussels collected from 17 sites in and around Halifax Harbour. Up to 80 of 159 CBs ranging from di- to heptachlorinated congeners were detected in mussels. The CB fingerprint suggested a mixture of sources, including Aroclors 1242, 1254 and 1260, in widely varying proportions. Relatively high concentrations of several less chlorinated CBs were found in animals collected from a narrow inlet, the Northwest Arm, with no present industrial sources of contamination, except for a marina next to a large municipal park. The 3,3'-dichlorobiphenyl detected in some of these samples represented nearly 20% of the total CBs detected. This dichlorobiphenyl was also detected in all the harbour mussels, but at a lower relative concentration. This non-*ortho* chlorinated congener is not predominant in Aroclor or Clophen mixtures (<0.05%, w/w) and is not generally examined in environmental studies. Less chlorinated CBs, such as 3,3'-dichlorobiphenyl and 3,3',5-dichlorobiphenyl have been suggested as by-products formed during the manufacturing of dyes (printing inks, paints, etc.). These by-products may represent an origin for these outlier CBs found in mussels. Further investigation is needed to determine possible sources and distribution of less chlorinated CBs found in marine animals collected near urban centers. Non-*ortho* chlorinated CBs of low chlorination, e.g. 4,4'-

dichlorobiphenyl, have some 3-methylcholanthrene type inducing activity, congeners substituted in both *para* positions and at least two *meta* positions have the greatest toxic potential, while to the best of our knowledge not enough is known about the toxicity of these non-*ortho* low chlorinated CBs.

Effect of Humic Substances on Cadmium Accumulation by Zebrafish Embryo and Larva.
K. Burnison[1], T. Meinelt[2], R. Playle[3], M. Pietrock[2] and C. Steinberg[2]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany; [3]Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Experiments were done to investigate factors influencing the accumulation of Cd into zebrafish (*Danio rerio*) embryo and larva. The accumulation of ^{109}Cd was affected by concentration, time, presence of dissolved organic material (DOM) and the developmental stage of the fish. Zebrafish eggs showed a steady increase in Cd-accumulation over an 8 h exposure. This rise was slightly decreased by DOM at concentrations up to 10 mg C/L. DOM concentrations over 15 mg C/L decreased Cd uptake significantly. Cd is mainly accumulated in the fish egg's outer shell or chorion and only small amounts pass the eggshell barrier and are found in the perivitelline liquid and embryo. The accumulation of the Cd was significantly lowered when DOMs were present in concentrations of 10 mg C/L. The DOM was isolated from a brown water marsh (LM) and a eutrophic pond (SP). Both samples of DOM at 10 mg/L reduced the Cd-accumulation in the perivitelline liquid, the embryo, and the chorion. Newly hatched larvae showed an accumulation related to their developmental stage. In the 4 h exposed larvae the accumulation of Cd is reduced by both DOMs, but to a greater degree by LM. In larvae exposed for 24 h, Cd accumulation was still reduced by the presence of LM, but not by SP. The increased uptake of Cd in older larvae may be a consequence of increased active Ca uptake, and therefore Cd uptake, in the growing embryo.

Differing Metabolitic Fate of PACs after Acute Exposure of Trout. J.D. Leonard and J. Hellou. Fisheries and Oceans Canada, Bedford Institute of Oceanography, Dartmouth, NS.

Trout were exposed, orally at nearly identical levels, to 8 individual polycyclic aromatic compounds (PACs), namely naphthalene, phenanthrene, pyrene, benzo[*a*]pyrene, carbazole, fluorene, dibenzothiophene and dibenzofuran. After 7 d, the gall bladders of the fish were removed and the bile analyzed by HPLC with fluorescence detection, using quantitation based on the parent compounds. UV-VIS spectra were acquired for detectable peaks, to confirm similarity to the parent compounds. Total levels of PAH derived compounds were highest for the pyrene exposure. Levels of metabolites in several of the exposures were 100-1000 times less than the pyrene. The number of peaks detected in the various exposures and their relative concentrations differed widely. The major peak in the bile from the pyrene exposure constituted more than 99% of the total signal while in the case of the phenanthrene there were six peaks, including phenanthrene itself, contributing significantly to the total. Based on parallel work involving the partitioning of peaks and on mass spectrometric analyses, the non-parental peaks can be divided into two groups: complex conjugates such as glucuronides and simpler compounds such as alcohols. The identification and quantification of these bile metabolites leads to a better understanding of how PAHs are metabolized and released under acute exposure conditions.

Mentum Deformities in Midge Larvae (Diptera: Chironomidae) downstream of Northeastern New Brunswick Metal Mines. E.O.Swansburg[1], J.J.H. Ciborowski[1] and W.L. Fairchild[2]. [1]University of Windsor, Department of Biological Sciences and Great Lakes Institute for Environmental Research, Windsor, ON: [2]Fisheries and Oceans Canada, Gulf Fisheries Centre, Moncton, NB.

Abstract

Chironomid mentum deformities are increasingly being used as an indicator of contaminant exposure in both laboratory and natural field populations. To assess the effects of metal exposure on chironomid populations, sites receiving mine drainage from five mines in Northeastern New Brunswick and five reference sites were sampled.

Concentrations of total Zn and associated metals were significantly higher ($p<0.05$) and chironomid taxa richness was significantly lower at mine drainage receiving sites than at reference sites ($p<0.05$). Replacement of taxa was not observed at mine drainage receiving sites, but rather fewer taxa made up the chironomid community. No significant difference in chironomid community composition was observed between mine drainage receiving and reference sites. Cluster analysis of chironomid relational abundances identified three groups of sites, one of which contained only mine drainage receiving sites. Other groups contained members from both mine drainage receiving and reference sites. The incidence of deformities did not differ significantly among sites. No significant relationship exists between the incidence of deformities and the length of operation of the mine facility.

Introduction

Assessing the effects of contaminant exposure on aquatic ecosystems has frequently employed the use of benthic invertebrates (Wiederholm, 1984; Ciborowski *et al.*, 1995). Midge larvae (Diptera: Chironomidae) are commonly used due to their ubiquitous distribution in freshwater aquatic ecosystems. Their relatively sessile nature and close association with contaminant accumulating substrates (sediment, organic material), allows chironomids to reflect the location and severity of contamination (Resh and Rosenberg, 1984).

Metal exposure can elicit a variety of responses in chironomid larvae, including mortality (Hatakeyama, 1988; Pascoe *et al.*, 1989; Harrahy and Clements, 1997), impaired growth and development (Wentsel *et al.*, 1977; Wentsel *et al.*, 1978; Timmermans and Walker, 1989; Heinis *et al.*, 1990; Timmermans *et al.*, 1992; Postma and Davids, 1995), and reduced reproductive success (Kosalwat and Knight, 1987; Postma *et al.*, 1994). These endpoints are difficult to assess in field populations and thus limit our ability to measure the effects of metal exposure.

Hamilton and Saether (1971) first proposed examining chironomid deformity levels to assess effects from contaminant exposure. A deformity is defined as any morphological feature that departs from normal configuration (Warwick 1988). Higher incidences of mouthpart deformities in chironomid larvae have been found in populations exposed to high concentrations of contaminants (Warwick 1990; Dickman *et al.*, 1992; Lenat, 1993; Bird, 1994; Bird *et al.*, 1995; Diggins and Stewart, 1998; Groenendijk *et al.*, 1998; Janssens de Bistohoven *et al.* 1998a; Janssens de Bisthoven *et al.*, 1998b; Janssens de Bisthoven *et al.*, 1998c). Background levels of deformities range from 0-5% (Bird, 1994; Hudson and Ciborowski, 1996; Burt, 1998), while levels in contaminated locations can reach as high as 83% (Warwick *et al.*, 1987; Burt, 1998).

The objectives of this study were to examine the incidence of chironomid mentum deformities in chironomids collected from streams receiving mine drainage and reference streams.

Chironomid community composition was also examined to detect differences between mine drainage receiving and reference populations. Mine drainage receiving sites are expected to have higher metal concentrations in the water, lower chironomid taxa richness, altered community composition and increased an incidence of deformed larvae than observed at reference sites.

Materials and Methods

Sampling Sites: This study was conducted in the watersheds of the Northwest Miramichi and the Nepisiguit Rivers, in Northeastern New Brunswick. The water chemistry of these rivers is typical of Canadian Shield rivers, exhibiting low conductivity, are well oxygenated, and poorly buffered. The substrate of these rivers are characterized by loose cobble, with occasional bedrock outcroppings and limited soft sediment. Generally, they are fast flowing, shallow, and support substantial salmonid populations (Atlantic salmon, Brook trout, etc.) (BEAK, 1998). The geology of this region forms a rich tapestry of rock types and landscapes, with massive sulphide deposits resulting in a long history of mining activity. Exploitation of these rocks types has occurred throughout this century, with more intensive activity within the last 50 years. Historically, mining effluent and site drainage have resulted in high metal loadings and periodic episodes of low pH in streams and rivers draining this area (BEAK 1998).

Chironomid larvae were sampled from 5 streams receiving drainage from metal mining facilities (Heath Steele Mine, Stratmat Mine, Brunswick Mining and Smelting (No. 6), Caribou Mine and Wedge Mine) (Table 1). All sites sampled received untreated acid mine drainage (AMD) from the extraction of Zn, Cu, Pb and Ag concentrates at each of these facilities. The history of operation varies for each mine. Two mines have a history of operation exceeding 15 years (Heath Steele and Brunswick No. 6), 2 had operations ranging from 5-10 years (Caribou and Stratmat), and one was in operation for <5 years (Wedge) (Table 1).

Five reference sites were also sampled (Table 1). Each reference site was paired to one of the mine drainage receiving sites, each chosen due to their similarity in size, flow, and substrate composition specific to the mine drainage receiving sites.

Water Sampling and Analysis: Water samples (two per site) were collected using acid-washed polyethylene bottles at a depth equidistant from the surface of the water and the surface of the substrate. One water sample from each site was filtered through a 0.45 μm membrane filter to remove particulate matter. All water samples were acidified to a 2% HNO_3 (analytical grade) solution within 48 h of collection and stored at 4°C prior to analysis. Total and dissolved metal concentrations were analyzed by inductively coupled plasma equipped with an optical emission spectrophotometer (ICP-OES) at the University of Windsor, Great Lakes Institute for Environmental Research Analytical Laboratory.

Chironomid Collection: Chironomid larvae were collected throughout the month of June, 1999 using a modified Hess sampler (253-μm mesh size) and a D-shaped kick net (1-mm mesh size). Specimens were hand-picked in the field to ensure the collection of 200 individuals at each site. A minimum of 125 chironomids of one taxon is required to detect a doubling of the background level of deformities of 3- 6% as statistically significant at α=0.05 (Hudson and Ciborowski, 1996). Larvae were blotted on paper towels and preserved in chilled Carnoy's solution (3 parts anhydrous ethanol: 1 part glacial acetic acid). Remainder of benthic samples were preserved in Kahle's solution. Larvae were stored at 4°C prior to analysis.

Table 1. Location, sampling dates, and codes of reference and metal receiving sites

RIVER	LATITUDE, LONGITUDE	SAMPLING DATE	NO. MOUNTED LARVAE	CODE	LENGTH OF OPERATION
Reference Sites					
NW Miramichi	47°11.184, 65°53.671	June 4, 1999	212	HEA_R	
McCormack Brook	47°17.574, 66°06.512	June 9, 1999	204	STR_R	
S Branch Pabineau	47°26.474, 65°49.784	June 14, 1999	204	BRU_R	
Nepisiquit	47°23.502, 66°11.104	June 12, 1999	179	WED_R	
44 Mile Brook	47°23.545, 66°10.959	June 17, 1999	197	CAR_R	
Metal Receiving Sites					
Little S Tomogonops	47°17.538, 66°03.123	June 10, 1999	218	HEA_M	1955 - (33 years)
Mosquito Brook	47°19.012, 66°06.470	June 8, 1999	219	STR_M	1989 - 1993 (5 years)
Austin Brook	47°23.823, 65°49.165	June 11, 1999	197	BRU_M	1966 - 1983 (18 years)
Nepisiquit	47°23.652, 66°07.799	June 12, 1999	197	WED_M	(<5 years)
40 Mile Brook	47°24.064, 66°07.885	June 16, 1999	202	CAR_M	1970 - 1998 (8 years)

Chironomid Identification and Deformity Screening: The heads of individual chironomid larvae were severed and slide-mounted ventral side up with the severed body in CMC-9AF® aqueous mounting medium (Masters Company Inc., Bensenville, IL). Chironomid larvae were identified to the genera level according to Oliver and Roussell (1983), Wiederholm (1983), and Ferrington and Coffman (1996). Ten percent of the identified larvae were randomly selected and re-examined to ensure correct designation.

Absolute mentum deformities (a missing or additional tooth (Hudson and Ciborowski, 1996a)) were scored during larval identification, and re-examined to ensure correct designation. One person performed all examinations for deformities. The individuals displaying broken, chipped or worn teeth were classified as damaged, not deformed. Deformities in other structures were not examined. Data was expressed as proportion deformed, with standard error determined from the binomial distribution.

Statistical Analysis: Trace elemental concentrations (9 elements) in water and water quality data were analyzed by principal component analysis ($\log_{10}$ values). Factor scores were then analyzed using a paired comparison t-test ($p<0.05$) to test for differences between reference ($n=5$) and mine drainage receiving ($n=5$) sites.

Taxa richness was calculated as the number of different chironomid genera present at each sampling site. Differences in mean richness between mine drainage receiving and reference sites were analyzed using a paired comparison t-test on log_{10} transformed data ($p<0.05$).

For community analysis, chironomid composition data was expressed as a proportion of larvae examined at each site, and transformed into octaves. Data were summarized using principal component analysis. Factor scores were then analyzed using a paired comparison t-test ($p<0.05$) to test for differences between mean factor scores at mine drainage receiving (n=5) and reference (n=5).

Cluster analysis (Euclidean distance) was used to group the sampling sites according to community composition. Data was transformed into octaves. Incidence of deformities were analyzed by the percent of deformities per site, per subfamily, and per genera at each site. Paired comparison t-tests ($p<0.05$) were used to assess differences in reference and mine drainage receiving sites. A replicated goodness of fit test (G-statistic) was used to test the heterogeneity of total deformities ($p<0.05$) at all sampling sites. All data were log transformed to stabilize variances and improve normality of the data. All statistical analysis was performed using STATISTICA (StatSoft '98 Edition, Tulsa, OK).

Results

Water Quality and Trace Elemental Analysis: Ag, As, B, Be, Bi, Cd, Co, Cr, Cu, Mo, Ni, Pb, Sn, Ti, and V were undetected in all water samples. Maximum values of the remaining elements were generally highest at mine drainage receiving sites and minimum values at reference sites (Table 2).

Table 2. Mean (±1 SE) total water concentrations of trace elements (μg/L) at metal receiving and reference sites.

TRACE ELEMENT	**CONCENTRATION (μg/L)**	
	Reference Sites	Metal Receiving Sites
Aluminum (Al)	ND[1]	283.4 (92.4, n=3)
Barium (Ba)	3.95 (0.59)[a]	4.55 (0.75)[a]
Calcium (Ca)	4281 (646)	5298 (2070)
Iron (Fe)	84.9 (16.9)[a]	152.9 (50.4)[a]
Potassium (K)	402.7 (20.7)[a]	516.1 (38.5)[a]
Magnesium (Mg)	904 (85)	1051 (148)
Manganese (Mn)	15.7 (10.6)[a]	86.6 (40.7)[a]
Sodium (Na)	2088 (635)	1669 (157)
Strontium (Sr)	19.43 (3.36)	19.59 (4.16)
Zinc (Zn)	7.3 (3.3)[a]	117.7 (37.3)[a]

[1] not detected
[a] significantly different at $p<0.05$. Statistical analysis was by factor analysis (PCA) followed by a paired t-test.

Two significant factors were extracted from trace element concentrations at each site by PCA, accounting for 69.3% of the variance in the original data (Table 3). Concentrations of Ca, Mg, Na, and Sr loaded most heavily on the first factor, which did not significantly differ between mine drainage receiving and reference sites. Fe, Mn, and Zn loaded most heavily on the second factor, and were significantly higher at mine drainage receiving sites ($p<0.02$) than at reference sites (Fig 1). Ba and K did not load significantly on either factor.

Table 3 Identification of the elements loading most heavily on the first two principal components and the proportion of variance explained by each component.

Element	Factor 1	Factor 2
Barium	0.2123	0.3679
Calcium	**0.9387**	-0.1626
Iron	-0.2406	**0.7474**
Potassium	-0.1338	0.6754
Magnesium	**0.9174**	0.3039
Manganese	0.2488	**0.9248**
Sodium	**0.7352**	0.1868
Strontium	**0.9804**	-0.0834
Zinc	0.2105	**0.7858**
Proportion of Total Variance Explained	0.3835	0.3093

Chironomid Community Composition: A total of 2048 chironomid larvae were collected at 10 sites, belonging to 36 genera within 5 subfamilies (Table 4). Chironominae comprised 44%, Orthocladiinae 36%, Tanypodinae 16%, Diamesinae 3% and Pseudochironominae <1% of collected larvae. *Polypedilum* sp. was the most abundant genus, representing 25% of chironomid larvae collected. Some genera of *Cricotopus* and *Orthocladius* were difficult to distinguish and thus were classed jointly (i.e., Cricotopus/Orthocladius).

Community composition between mine drainage receiving and reference sites differed. *Thienemannimyia*, *Paratendipes*, and *Rheotanytarsus* were more abundant at reference sites, and *Eukiefferiella*, *Cricotopus*, and *Cricotopus/Orthocladius* were more abundant at mine drainage receiving sites. *Phaenopsectra* and *Synorthocladius* were absent at all mine drainage receiving sites, while there were no taxa unique to these sites. Chironomid taxa richness among reference and mine drainage receiving sites ranged from 7-22 genera. Mine drainage receiving sites had a mean taxa richness of 11 (±1 SE), which was significantly lower ($p<0.05$) than the mean number of taxa at reference sites [18 (±1 SE)] (Fig. 2).

Principal component analysis of the eight most abundant chironomid genera extracted three significant factors, accounting for 81.1% of the variance (Table 5). Relative abundance of *Cricotopus, Eukiefferiella,* and *Thienemannimyia* loaded most heavily on the first factor, proportions of *Polypedilum* and *Micropsectra* on the second factor, and *Paratendipes* on the third factor. *Cricotopus/Orthocladius* and *Larsia* did not load significantly on any factor (Fig. 3).

Paired comparison t-tests of factor scores revealed no significant difference in chironomid composition between mine drainage receiving and reference sites.

Three cluster were identified by cluster analysis. Sites HEA_M and BRU_M are member of the cluster A, HEA_R, WED_R, WED_M, STR_R, and STR_M in cluster B, and BRU_R, CAR_R, and CAR_M in the cluster C (Fig. 4). *Eukiefferiella* and *Cricotopus* were most typical of cluster A communities. Cluster B was typified by the presence of *Larsia*, *Thienemannimyia*, *Paratendipes*, *Cricotopus/Orthocladius*, and *Rheotanytarsus*. Composition of cluster C consisted of lower relative abundance of *Polypedilum* and *Micropsectra*.

Chironomid Deformities: A total of 22 deformed individuals were observed in chironomid samples, from 9 different genera. Arithmetic mean percentage (± SE) of total deformed individuals was 1.37 (0.27) in larvae from mine drainage receiving sites and 0.78 (0.25) in larvae from reference sites. The difference was not statistically significant (Fig. 5). Nor were differences noted when individual subfamilies or genera were examined. There was no significant heterogeneity in the distribution of deformities among the 10 sampling sites (G-statistic test for heterogeneity $p<0.05$). There was no significant relationship between the mean percentage of total deformed individuals and the length of operation of the mine facility (Fig. 6).

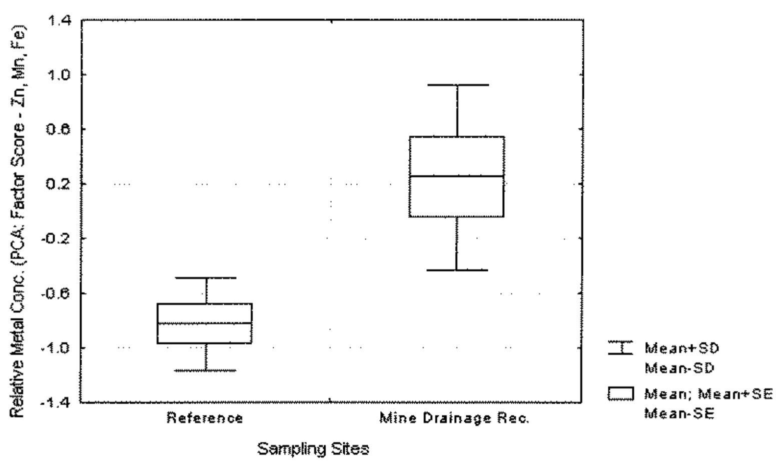


Fig. 1 Factor scores from principal component analysis, of relative metal concentrations (μg/L) in water samples from reference and mine drainage receiving sites.

Discussion

Benthic community structure in streams is a reflection of relative health of the ecosystem. However, the examination of community parameters may be less sensitive to contaminant exposure as well as being more difficult to interpret, in terms of change in community structure and function (Wiederholm 1984). The examination of population and individual parameters, such as deformities, may serve as a more sensitive biomarker, allowing the effects of contaminant exposure to be readily assessed (Wiederholm 1984).

Field studies have shown that metal exposure reduces the abundance and species richness of aquatic insects and alters the proportional abundance of different groups (Wiederholm 1984). Richness was significantly reduced at mine drainage receiving sites, suggesting that not all taxa were equally adept at tolerating metal exposure. Winner et al. (1980) concluded that taxa richness is a sensitive indicator of metal exposure.

Table 4. Number of chironomids and deformities in mine receiving and reference sites

	Reference Sites					Metal Receiving Sites				
	HEA_R	STR_R	BRU_R	WED_R	CAR_R	HEA_M	STR_M	BRU_M	WED_M	CAR_M
Tanypodinae										
Ablabesmyia			14		9			1		
Larsia		2	35	21	13		2		2	35
Paramerina			1		1					
Thienemannimyia	5	6	11 (1)	31 (1)	90 (1)		4		10	26 (1)
Macropelopia			1	1			1			1
Nilotanypus		1	1				3			
Procladius			1							
Diamesinae										
Diamesa		1				5	4	1		22
Pagastia	1									
Potthastia	8			7						14
Chironominae										
Chironomini										
Cryptochironomus	1		5	1		1				

	Reference Sites					Metal Receiving Sites				
	HEA_R	STR_R	BRU_R	WED_R	CAR_R	HEA_M	STR_M	BRU_M	WED_M	CAR_M
Demicryptochironomus	1		1	1				1		
Microtendipes	6	15	9	4					1	
Nilothauma	3									
Parachironomus		1								
Paralauterborniella	1			2						
Paratendipes	14	7	30 (1)	5	2		2		2	1
Phaenopsectra			1 (1)	16						
Polypedilum	55	90	25	86 (1)	12	1	97 (2)	34 (2)	104 (2)	
Tanytarsini										
Cladotanytarsus			1							
Micropsectra	16	11		2	15		35	1	21	
Paratanytarsus			3							
Rheotanytarsus	21	19	43				1	1		
Stempellinella		1			3					
Tanytarsus	12	6	2	12	7		17 (1)	1		
Pseudochironominae										
Pseudochironomus	2									
Orthocladiinae										
Eukiefferiella	3	12	11		5	85 (2)	25	71	20	
Corynoneura					5			2		
Cricotopus	6		2	17	2	116 (1)	9	38 (1)	5	22
Cricotopus/Orthocladius	17	1	1	7	2 (1)	7 (1)		26	3	52

	Reference Sites					Metal Receiving Sites				
	HEA_R	STR_R	BRU_R	WED_R	CAR_R	HEA_M	STR_M	BRU_M	WED_M	CAR_M
Lopescladius					2					
Orthocladius	23			1					1	1
Parakiefferiella	1									
Parametriocnemus	3	8	2		11		8	7	7	
Psectrocladius	2							6 (1)		27
Synorthocladius		5			13					
Thienemanniella	3	15 (1)	1		5	1	8	4		
Sum	212	204	204	216	197	218	219	197	179	202
Taxa Richness	22	17	22	16	17	7	14	15	11	10
Proportion Deformed	0.00	0.49	1.47	0.93	1.02	1.83	1.37	2.03	1.12	0.50

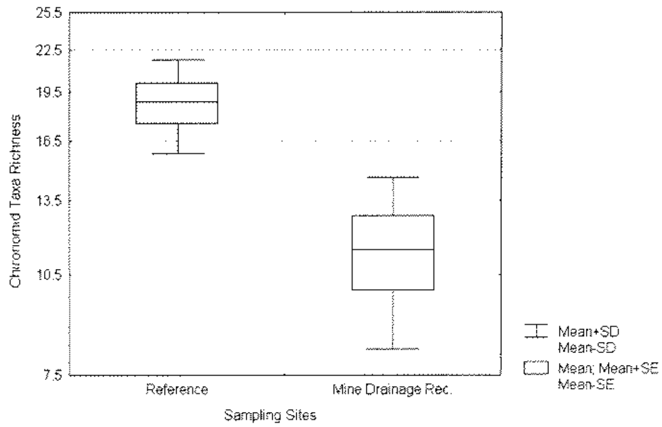
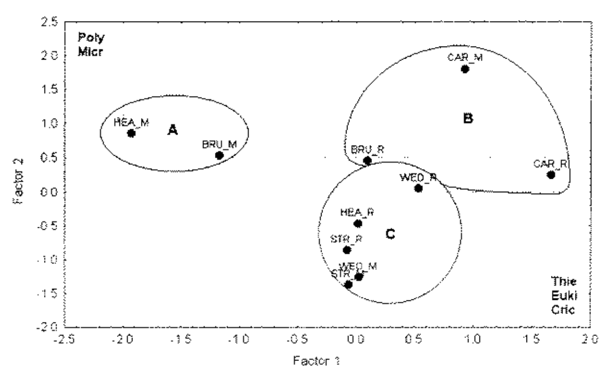


Fig. 2. Chironomid taxa richness at reference and mine drainage receiving sites.

Table 5 Identification of the relative abundances of chironomid taxa loading most heavily on the first three principal components and the proportion of variance explained by each component.

Element	Factor 1	Factor 2	Factor 3
Larsia	0.5416	0.5927	-0.4770
Thienemannimyia	**0.7844**	0.2141	0.3241
Paratendipes	0.1153	-0.0244	**-0.9528**
Polypedilum	0.0335	**-0.8917**	-0.0579
Micropsectra	0.2274	**-0.8362**	0.2936
Eukiefferiella	**-0.8760**	0.1321	0.3135
Cricotopus	**-0.7489**	0.4737	0.3280
Cricotopus/Orthocladius	0.0646	0.6884	0.1940
Proportion of Total Variance Explained	0.2884	0.3260	0.1967



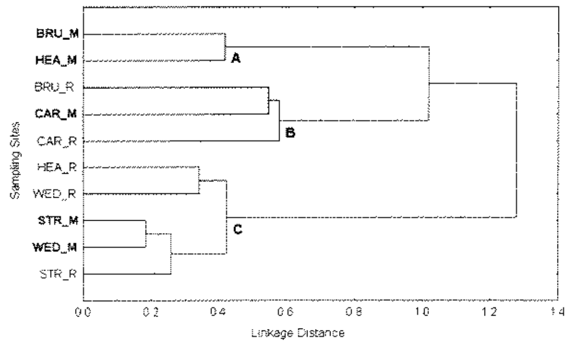


Fig. 3 Factor scores from principal component analysis of relative abundance of *Larsia*, *Thienemannimyia* (Thie), *Paratendipes*, *Polypedilum* (Poly), *Micropsectra* (Micr), *Eukiefferiella* (Euki), and *Cricotopus* (*Cric*) at reference (Mine_R) and mine drainage receiving (Mine_M) sites. Grouping according to cluster analysis.

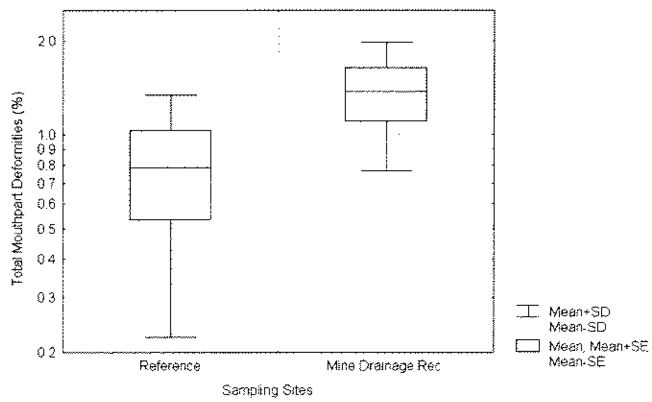


Fig. 4 Cluster analysis (Ward's linkage) of chironomid relative abundances at reference (Mine_R) and mine drainage receiving (Mine_M) sites. Three grouping identified as A, B, and C.

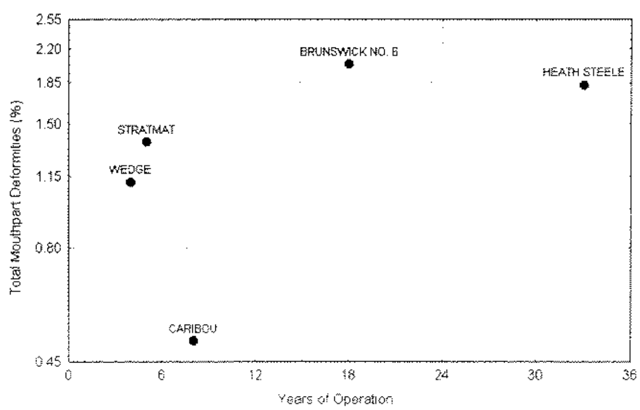


Fig. 5 Total mouthpart deformities (percentage) in chironomids collected at reference and mine drainage receiving sites.

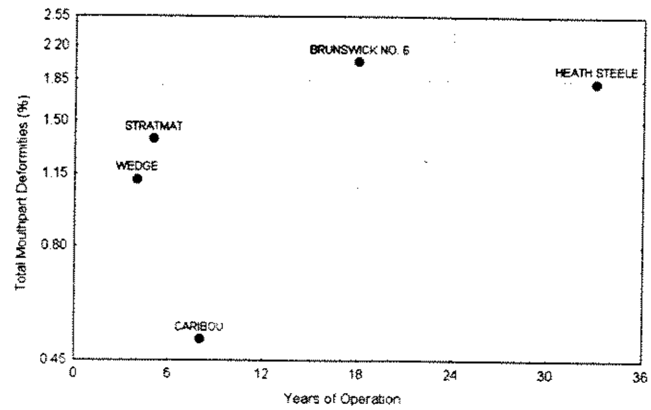


Fig. 6 Relationship between total mouthpart deformities (percentage) in chironomids collected at mine drainage receiving sites and the number of years of operation of the mining facility

Community composition shifted towards a greater abundance of tolerant individuals and a loss of sensitive taxa. Reduced abundance of *Thienemannimyia*, *Paratendipes*, and *Rheotanytarsus* and the absence of *Phaenopsectra* and *Synorthocladius* at mine drainage receiving sites suggests that these genera are more sensitive to metal exposure. Three Orthocladiinae genera, *Eukiefferiella*, *Cricotopus*, and *Cricotopus/Orthocladius*, were more abundant at mine drainage receiving sites. Their presence at these sites, supports previous research suggesting that they have an increased ability to tolerant higher concentrations of metal (Yasuno *et al.*, 1985, Armitage, 1980, La Point *et al.*, 1984, Chadwick *et al.*, 1986, Clements *et al.*, 1988). Despite these trends, differences in abundances of the eight most common genera was not statistically significantly. This could be attributed to the qualitative nature of the sampling, rather than attempting to determine actual densities of these genera at all sampling sites.

While the percentage of deformed individuals was generally higher at mine drainage receiving sites, no significant difference was observed. Incidences of deformities comparable to those observed in reference population have been observed in metal exposed populations examined by Bird *et al.* (1995), Postma *et al.* (1995), and Janssens de Bisthoven *et al.* (1998b). Inadequate sample size could result in actual deformities levels being incorrectly reported. Hudson and Ciborowski (1996) suggested that to detect a doubling of the background incidence level of 3% to 6%, 125 chironomids of one taxa would be required. In none of the sampling sites, were 125 chironomids of one genus collected. *Polypedilum* was the most abundant genera, however, average number collected in reference and mine drainage receiving sites did not approach 125. The trend of increased numbers of deformed individuals at mine drainage receiving sites would suggest that improving the power of the analysis may have resulted in a statistical difference. However, the difference in the incidence of deformities is rather small considering what has been observed in other populations (<1.00%). This seems to suggest that incidences of deformities are lower than would be expected.

Tolerance to metal exposure has been observed in many aquatic organisms (Roesijadi 1992). Metal tolerance has been observed in midge larvae (Wentsel et al., 1978; Krantzberg and Stokes, 1989; Gerhardt and Janssens de Bisthoven, 1995). Midge larvae populations chronically metal exposed exhibit tolerance (Postma et al. (1995), altering their response to biomonitoring indices. Chironomids living in a metal polluted Belgium river showed lower deformity frequencies (Janssens de Bisthoven et al., 1998b). Lower frequencies of deformities at mine drainage receiving sites could be explained by tolerance acquisition through chronic exposure to metals.

Cluster analysis identified three groupings according to the chironomid community composition. While, two of the mine drainage receiving sites formed one group (A), three other mine sites were similar in chironomid community composition to reference sites. This would suggest that Heath Steele and Brunswick No. 6 Mines are more impacted by mine drainage, while the community composition of the remaining mines does not reflect a significant mine drainage impact. Differences in habitat and concentrations of metals could account for these difference in groupings.

Mine drainage exposed sites exhibited elevated metal concentrations and significantly reduced taxa richness, possibly altering form and function of the community. The lack of difference in deformity levels may suggest tolerance, due to prolonged metal exposure. This may indicate that the examination of deformities in areas of prolonged metal exposure may be of little value in assessing water quality.

References

Armitage, P.D. 1980. The effects of mine drainage and organic enrichment on benthos in the River Nent System, Northern Pennies. Hydrobiologia 74: 119-128.

BEAK International Incorporated. 1998. 1997 Field Program - AETE Heath Steele Draft Site Report. Aquatic Effects Technology Evaluation Program, Canadian Centre for Mineral and Energy Technology (CANMET), Natural Resources Canada, Ottawa, Ontario

Bird, G.A. 1994. Use of chironomid deformities to assess environmental degradation in the Yamaska River, Quebec. Environ. Monit. Ass. 30: 163-175.

Bird, G.A., W.J. Schwartz and D.L. Joseph. 1995. The effect of 210 Pb and stable lead on the induction of menta deformities in *Chironomus tentans* larvae and on their growth and survival. Environ. Toxicol. Chem. 14: 2155-2130.

Burt, JA. 1998. Deformities and Fluctuating Asymmetry in Chironomidae (Diptera): baselin and stress-induced occurrence. Masters thesis, University of Windsor, Windsor, ON.

Ciborowski, J.J.H., L.D. Corkum and L.A. Hudson. 1995. The use of benthic invertebrates fro monitoring contaminated sediments. Eighth Annual NABS Technical Information Workshop, Keystone, Colorado.

Chadwick, J.W., S.P. Canton and R.L. Dent. 1986. Recovery of benthic invertebrate communities in Silver Bow Creek, Montana, following improved metal mine wastewater treatment. Water Air Soil Pollut. 28: 427-438.

Clements, W.H., D.S. Cherry and J. Cairns. 1988. The impact of heavy metals on macroinvertebrate communities: A comparison of observational and experimental results. Can. J. Fish. Aquat. Sci. 45: 2017-2025.

Dickman, M., A. Brindle and M. Benson. 1992. Evidence of teratogens in sediments of the Niagara River watershed as reflected by chironomid (Diptera: Chironomidae) deformities. J. Great Lakes Res. 18: 467-480.

Diggins, T.P., and K.M. Stewart. 1998. Chironomid deformities, benthic community composition, and trace elements in the Buffalo River (New York) Area of Concern. J. N. Am. Benthol. Soc. 17: 311-323.

Ferrington L.C., and W.P. Coffman. 1996. Chironomidae. *In*: An introduction to the Aquatic insects of North America, 3rd Edition, p. 635-754. *Edited by* Merritt R.W., and Cummins K.W. Kendall Hunt Publishing Co., Dubuque..

Gerhardt, A., and L. Janssens de Bisthoven. 1995. Behavioural, developmental and morphological responses of *Chironomus* gr. *thummi* larvae (Diptera, Nematocera) to aquatic pollution. J. Aquat. Ecosyst. Health 4: 205-214.

Groenendijk, D., L.W.M. Zeinstra and J.E. Postma. 1998. Fluctuating asymmetry and mentum gaps in populations of the midge *Chironomus riparius* (Diptera: Chironomidae) from a metal-contaminated river. Environ. Toxicol. Chem. 17: 1999-2005.

Hamilton, A.L., and O.A. Saether. 1971. The occurrence of characteristic deformities in the chironomid larvae of several Canadian lakes. Can. Entomol. 103: 363-368.

Harrahy, E.A., W.H. and Clements. 1997. Toxicity and bioaccumulation of a mixture of heavy metals in *Chironomus tentans* (Diptera: Chironomidae) in synthetic sediment. Environ. Toxicol. Chem. 16: 317-327.

Hatakeyama, S. 1988. Chronic effects of Cu on reproduction of *Polypedilum nubifer* (Chironomidae) through water and food. Ecotox. Environ. Saf. 16: 1-10.

Heinis, F., K.R. Timmermans and W.R. Swain. 1990. Short-term sublethal effects of cadmium on the filter feeding chironomid larva *Glyptotendipes pallens* (Meigen) (Diptera). Aquat. Toxicol. 16: 73-86.

Hudson, L.A., and J.J.H. Ciborowski. 1996a. Spatial and taxonomic variations in incidence of mouthpart deformities in midge larvae (Diptera, Chironomidae, Chironomini). Can. J. Fish. Aquat. Sci. 53: 297-304.

Janssens de Bisthoven, L., P. Nuyts, B. Goddeeris and F. Ollevier. 1998a. Sublethal parameters in morphologically deformed *Chironomus* larvae: clues to understanding their bioindicator value. Freshwater Biol. 39: 179-191.

Janssens de Bisthoven, L., J.F. Postma, P. Parren, K.R. Timmermans and F. Ollevier.. 1998b. Relations between heavy metals in aquatic sediments and in *Chironomus* larvae of Belgian lowland rivers and their morphological deformities. Can. J. Fish. Aquat. Sci. 55: 688-703.

Janssens de Bisthoven, L., A. Vermeulen and F. Ollevier. 1998c. Experimental induction of morphological deformities in *Chironomus riparius* larvae by chronic exposure to copper and lead. Arch. Environ. Contam. Toxicol. 35: 249-256.

Kawai, K., T. Yamagishi, K. Kubo and K. Konishi. 1989. Usefulness of chironomid larvae as indicators of water quality. Jap. J. Sanit. Zool. 40: 269-283.

Kosalwat, P., and A.W. Knight. 1987. Chronic toxicity of copper to a partial life cycle of the midge, *Chironomus decorus*. Arch. Environ. Contam. Toxicol. 16: 283-290.

Krantzberg, G., and P.M. Stokes. 1989. Metal regulation, tolerance and body burdens in the larvae of the genus Chironomus. Can. J. Fish. Aquat. Sci. 46: 389-398.

La Point, T.W., S.M. Melanson and M.K. Morris. 1984. Relationships among observed metal concentrations, criteria, and benthic community structural responses in 15 streams. J. Water Pollut. Control Fed. 56: 1030-1038.

Lenat, S.R. 1993. Using mentum deformities of *Chironomus* larvae to evaluate the effects of toxicity and organic loading in streams. J. N. Am. Benthol. Soc. 12: 265-269.

Oliver, D.R., and M.E. Roussell. 1983. The Insects and Arachnids of Canada, Part II: The genera of larval midges of Canada, Diptera: Chironomidae. Agriculture Canada, Ottawa, pp. 263.

Pascoe, D., K.A. Williams and D.W.J. Green. 1989. Chronic toxicity of cadmium to *Chironomus riparius* Meigen - effects upon larval development and adult emergence. Hydrobiologia 175: 109-115.

Postma, J.F., M.C. Buckert-de-Jong, N. Staats and C. Davids.. 1994. Chronic toxicity of cadmium to *Chironomus riparius* (Diptera: Chironomidae) at different food levels. Arch. Environ. Contam. Toxicol. 26: 143-148.

Postma, J.F., and C. Davids. 1995. Tolerance induction and life cycle changes in cadmium exposed *Chironomus riparius* (Diptera) during consecutive generations. Ecotox. Environ. Saf. 30: 195-202.

Postma, J.F., M. Kyed and W. Admiraal. 1995. Site-specific differentiation in metal tolerance in the midge *Chironomus riparius* (Diptera, Chironomidae). Hydrobiologia 315: 159-165.

Roesijadi G. 1992. Review. Metallothioneins in metal regulation and toxicity in aquatic animals. Aquat. Toxicol. 22: 81-114.

Resh V.H., and D.M. Rosenburg. 1984. The ecology of aquatic insects. Praeger Publishers, New York, NY.

Timmermans, K.R., and P.A. Walker. 1989. The fate of trace metals during the metamorphosis of Chironomids (Diptera, Chironomidae). Environ. Pollut. 62: 73-85.

Timmermans, K.R., W. Peeters and M. Tonkes. 1992. Cadmium, zinc, lead and copper in *Chironomus riparius* (Meigen) larvae (Diptera, Chironomidae): uptake and effects. Hydrobiologia 241: 119-134.

Warwick, W.F. 1988. Morphological deformities in Chironomidae (Diptera) larvae as biological indicators of toxic stress, p. 281-320. *In*: Toxic contaminants and ecosystem health: a Great Lakes focus. Advances in Envionmental Science and Technology, Vol. 21. *Edited by* M.S. Evans. John Wiley & Sons, New York, NY.

Warwick, W.F. 1990. Morphological deformities in Chironomidae (Diptera) larvae from the Lac St. Louis and LaPierre basins of the St. Lawrence River. J. Great Lakes Res. 16: 185-208.

Warwick, W.F., J. Fitchko, P.M. McKee, D.R. Hart and A.J. Burt. 1987. The incidence of deformities in *Chironomus* spp. from Port Hope Harbour, Lake Ontario. J. Great Lakes Res. 13: 88-92.

Wentsel, R., A. McIntosh and G. Atchinson. 1977. Sublethal effects of heavy metal contaminated sediment on midge larvae (*Chironomus tentans*). Hydrobiologia 56: 153-156.

Wentsel, R., A. McIntosh and G. Atchinson. 1978. Emergence of the midge *Chironomus tentans* when exposed to heavy metal contaminated sediment. Hydrobiologia 57: 195-196.

Wiederholm, T. 1983. Chironomidae of the Holarctic region, Keys and diagnoses, Part I: Larvae. Entomologica Scandinavica, Supplement No. 19.

Wiederholm, T. 1984. Responses of aquatic insects to environmental pollution, p. 508-557. *In*: The ecology of aquatic insects. *Edited by* V.H. Resh and D.M. Rosenburg. Praeger Publishers, New York, NY.

Winner, R.W., J.S. Van Dyke, N. Caris and M. Farrel. 1975. Response of the macroinvertebrate fauna to a copper gradient in an experimentally-polluted stream. Verh. Int. Ver. Limnol. 19: 2121-2127.

Vermeulen, A.C., P.C. Dall, C. Lindegaard, F. Ollevier and B. Goddeeris. 1998. Improving the methodology of chironomid deformation analysis for sediment toxicity assessment: a case study in three Danish lowland streams. Arch. Hydrobiol. 144: 103-125.

Yasuno, M., S. Hatakeyama and Y. Sugaya. 1985. Characteristic distribution of chironomids in the Rivers polluted with heavy metals. Verh. Int. Ver. Limnol. 22: 2371-2377.

Early Mortality Syndrome and Tissue-specific Apoptotic Cell Death during Embryonic Development in Lake Trout (*Salvelinus namaycush*) **from Lake Michigan.** J.J. Whyte, J.A. Allert and D.E. Tillitt. Columbia Environmental Research Center (CERC) BRD, USGS, Columbia, MO.

The gross pathological symptoms of early mortality syndrome (EMS) in Great Lakes salmonids with low thiamine concentrations in their eggs resemble those seen in fish embryos exposed to planar halogenated hydrocarbons (PHHs). Programmed cell death (apoptosis) in the embryonic vasculature, neural and digestive tissues during development has been observed in Atlantic salmon (*Salmo salar*) from the New York Finger Lakes experiencing Cayuga syndrome and from the Baltic Sea exhibiting M74. These disorders are highly similar to EMS. This same tissue-specific pattern of apoptosis has also been observed in Medaka (*Orizias latipes*) embryos exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. These results indicate that apoptotic cell death in specific embryonic tissues may result from disruption of a pathway common to both thiamine utilization and PHH metabolism. The present study examines the temporal and tissue-specific nature apoptotic cell death during development in lake trout (*Salvelinus namaycush*) from Lake Michigan. Trout from this region exhibit low egg thiamine levels, elevated PHH concentrations, and a high incidence of EMS. Lake trout eggs from both feral (Lake Michigan) and laboratory sources were spawned and reared in laboratory culture conditions. Developing embryos were monitored for expression of clinical signs of EMS (loss of equilibrium, lethargy, erratic swimming, hyperexcitability). At several critical stages of development, embryos were collected and preserved for apoptotic and histological analysis of tissue sections. Our preliminary results reveal that apoptotic cells are readily apparent in gill and neural tissue of developing lake trout from Lake Michigan, supporting the previous laboratory and field investigations. By examining the temporal relationship between the incidence of tissue-specific apoptotic cell death and the onset of EMS, our studies will isolate the early stages of toxicity that occur prior to gross pathological effects. Results will be related to thiamine and PHH concentrations in eggs and tissue-specific induction of CYP1A activity, an indicator of PHH exposure. Determining if the pathological symptoms of EMS are preceded by programmed cell death in critical tissues during development will contribute greatly to an understanding the cellular etiology of this disorder.

The Effect of Lindane on Tadpole Growth and Development. K. Serben and D. Forsyth. Environment Canada, Canadian Wildlife Service, Saskatoon, SK.

Recent reports of high numbers of deformities in frogs and global amphibian declines have researchers concerned. Pesticides are among the possible causes for these trends. Amphibians are sensitive to aquatic pollution due to their permeable skin and biphasic lifestyle. Lindane, a component of Vitavax® is frequently found in prairie ponds, due to its extensive use as a seed treatment for canola in Saskatchewan. To test the effect of lindane on tadpole growth and development, wood frog tadpoles were raised in outdoor microcosms until metamorphic climax. Four concentrations of lindane and one control were used: 0.1 and 1.0 µg/L (actual concentrations of lindane found in prairie ponds), 10 µg/L (the highest calculated concentration that could occur in ponds), and 100 µg/L (a high concentration for comparison). Time to metamorphosis, length and weight at metamorphosis, and survival were compared among treatments. Following a standardized acute stress protocol, blood was collected from newly metamorphosed animals for corticosterone and thyroid hormone analyses. Water samples were collected periodically throughout the experiment for lindane residue analysis and analyses of water quality parameters such as ammonia concentration, nitrate/nitrite levels, conductivity, hardness, pH, and alkalinity. Water samples were also collected from ponds in three agricultural fields, two wild sites and one pasture in order to evaluate how well the microcosms mimic field conditions. Length and weight at metamorphosis of froglets in three of these ponds were also compared to froglets in the microcosms.

The Toxicity of Copper Sulphate to Plants in Artificial and Field-collected Reference Soils. G. Stephenson[1], N. Feisthauer[2], N. Koper[2], J. Qiu[2], J. McCann[2] and R. Scroggins[3]. [1]University of Guelph, Guelph, ON; [2]ESG International Inc., Guelph, ON; [3]Environment Canada, Ottawa, ON.

A series of toxicity tests with copper sulphate are currently being conducted to determine if the test methods developed for organic contaminants are applicable to toxicity assessment of metal compounds. Acute toxicity tests were used to identify the concentrations of copper sulphate in artificial soil (AS) and a field-collected reference soil (RS) that resulted in effects that bracketed the response range (no observable to adverse) in seven species of plants. The results of these screening tests were then used to identify the range of exposure concentrations that would be optimal for definitive (e.g., longer-term) plant toxicity tests designed to estimate the EC_{20} and EC_{50} values using non-linear regression procedures. The measurement endpoints for both the screening and definitive tests included seedling emergence, root and shoot length, root and shoot wet and dry mass, and the ratio of shoot to root. The results of the screening tests indicated that root length and root mass were the most sensitive measurement endpoints and that EC_{50} values for root length for the seven species of plants ranged from 2430-4873 mg $CuSO_4$/kg soil d.w. in the reference soil. Root lengths for barley (*Hordeum vulgare* var. Chapais) and northern wheatgrass (*Agropyron dasystachyum*) tested in artificial soil ranged from 1003-1170 mg $CuSO_4$/kg soil d.w. The copper equivalent concentrations were 968-1941 mg and 400-466 mg for the RS and AS, respectively. The plant species most sensitive to copper sulphate in both RS and AS was northern wheatgrass; whereas, carrot (*Daucus carota* var. Royal Chantenay) was minimally affected when exposed to similar levels of contamination in RS. Barley was relatively insensitive when exposed to copper sulphate in AS relative to the RS. These results suggest that the test methods developed earlier for assessing soils contaminated with petroleum hydrocarbons were equally applicable to soils contaminated with metals.

Risk Assessments and Evaluations

Reactive Chlorine Species - Environmental Behaviour, Aquatic Toxicity, and Canadian Water Quality Guidelines. U. Schneider. Environment Canada, Guidelines and Standards Division, Hull, QC.

The four main sources of reactive chlorine species (RCS, i.e., hypochlorous acid, organic and inorganic chloramines, and their bromine-derivatives) to the environment are treated wastewater effluents, chlorinated cooling water effluents, spills due to breaks in the drinking water distribution system, and uncollected releases of drinking water. In freshwater, the dominant species are hypochlorous acid or monochloramine. In marine or estuarine water, bromide ions (Br^-) cause rapid replacement of chlorine, forming hypobromous acid [HOBr], bromamines, and bromochloramines, collectively called chlorine-produced oxidants (CPO). RCS are chemically interconvertible and can co-occur in the environment; the respective dominant species is determined by site-specific conditions. The species-specific analytical determination, especially at the low levels of toxicological relevance (i.e., low microgram per litre concentrations), is difficult and sometimes not possible or practical due to the reactive nature and the complex chemistry of these compounds. Therefore, the development of separate guidelines for hypochlorous acid and monochloramine was deemed inappropriate. Instead, a Canadian water quality guideline (CWQG) of 0.5 µg/L for RCS was derived for the protection of freshwater life, and a guideline of 0.5 µg/L for CPO was derived for the protection of marine life. If chemical species-specific identification is available, the guideline applies to the sum of all reactive chlorine species. It should be noted that the lowest reliable detection limit currently is approximately 10 µg/L, a value that is higher than the CWQGs. Therefore, any detection of RCS is an indication that aquatic life is potentially being negatively affected. This presentation will summarize the relevant chemistry and toxicology, and will explain the development of the recent "Canadian Water Quality Guidelines for Reactive Chlorine Species."

Ecological Risk Assessment of Ammonia. F.A. Jensen, G.R. Craig, D.R.J. Moore and M.B. Constable. Environment Canada, Edmonton, AB.

Ammonia was ranked second among the substances released to the Canadian environment in 1994 by the National Pollutant Release Inventory. After review by a Ministerial Expert Advisory Panel, ammonia was included on a list of 25 priority substances requiring assessment under Canadian environmental legislation. The assessment process is outlined in this presentation with a focus on potential water quality effects using sub-lethal toxicity concentration–response data published in the public domain literature for different species of fish and invertebrates. The data are further analyzed to determine the risk of potential effects from a range of concentrations. The risk assessment is applied to case studies of ammonia contamination from sewage effluents in the North Saskatchewan River and Hamilton Harbour using effluent plume predictions using CORMIX and monitoring data, respectively. The conditions under which ammonia meets the criteria of "toxic" as defined by the Canadian Environmental Protection Act are illustrated and the possible consequences discussed.

Toxicity Assessment of Storm Water and Wastewater Collected From 8 Wing Trenton. L.J. Novak[1], K.E. Holtze[1] and L. Cocks[2]. [1]ESG International Inc., Guelph, ON; [2]National Defence, Air Command Headquarters, Winnipeg, MB.

The main objectives of this study were to: evaluate the suitability of the effluent monitoring program at 8 Wing Trenton (Ontario) to determine if it is adequate to predict whether the effluent is acutely lethal to rainbow trout; evaluate the adequacy and practicality of the Microtox® test to predict acute lethality of wastewater/storm water to rainbow trout; and determine if the current effluent monitoring program should include toxicity testing. Thirty-five of the 39 samples collected from five of the routine monitoring sites were non lethal to rainbow trout (0% mortality). Of the four samples lethal to trout, only two collected from the aircraft Hangar location resulted in significant mortality (≤80%). Trenton's environmental management practices at the study locations appear to adequately protect against acute lethality to rainbow trout. Additional data from toxic samples is required before it can be determined if the chemical monitoring program is adequate to predict whether the discharges will be acutely lethal to trout. Microtox® may be a suitable rapid screening test, however additional data is required since the majority of the samples tested were non lethal to trout. Consideration should also be given to a toxicity assessment of storm water and wastewater discharges at other DND bases.

Human Health Risk Assessment for a Former Wood Preserving Plant Site. A.G. Verbeek[1], G.A. Clyde[1], K.T. Himbeault[2] and J. Goodin[3]. [1]Conor Pacific Environmental Technologies Inc., Edmonton, AB; [2]#4-130 Robin Crescent, Saskatoon, SK; [3]2240 Speakman Drive, Mississauga,

ON.

A human health risk assessment was completed as part of a brownfield development project, to facilitate the remediation/risk management of a former wood preserving plant site, which had been in operation for 24 years until the late 1980s. During that time, the plant provided year round pressure impregnation of rail ties and large dimensional wood products with coal tar, a coal tar/creosote mixture, and small amounts of pentachlorophenol (PCP) in a petroleum oil carrier fluid. The south-central portion of the site (~5 hectares), which contained the wood treatment facility and rail lines, contains contaminated soil, DNAPL and the majority of the contaminated groundwater at excessive depth. The deeper contamination will remain at depth, as complete remediation to depth is not feasible. A human health risk assessment was completed for this area as a means to identify the measures necessary to protect public health during and after remediation. The human health risk assessment incorporated the input of a multi-stakeholder group consisting of local stakeholders, Alberta Environment and the Calgary Regional Health Authority.

The human health risk assessment indicated there would be no unacceptable risks to onsite or off-site persons associated with the migration of dust during remediation. Secondly, there would be no unacceptable risks to onsite users of the parkland and commercial buildings (without a basement) within the risk assessment area after the site is remediated to meet remedial objectives. Potential human health risks estimated for Polycyclic Aromatic Hydrocarbons (PAHs) based on dermal exposure were the most sensitive and therefore limiting. Remediation of the south-central portion of the site to protect human health through risk-based remedial objectives for soil, will be driven by the chosen land use, parkland, commercial or commercial with a daycare, and these remedial objectives will be 1.44, 2.58 or 1.77 mg/kg of Benzo[*a*]pyrene respectively.

Nutrient Loading in the Canadian Environment: Do We Have a Problem or Not? E.S. Roberts[1], R.A. Kent[2], P.A. Chambers[3], C. Gagnon[4] and P-Y. Caux[2]. [1]ESR Consulting, Nepean, ON; [2]Environment Canada, Guidelines and Standards Division, Ottawa, ON; [3]Environment Canada, National Hydrology Research Institute, Saskatoon, SK; [4]Environment Canada, Chemical Evaluation Division, Ottawa, ON.

Historically, nutrients such as P and N have been blamed for the eutrophication of water bodies, but more serious is the current threat that N-containing compounds are having on the health of aquatic and terrestrial organisms. Nutrients are elements essential to plant growth but in excess they are toxic to both plants and animals. Despite the clearly identified toxicity of certain nitrogen forms, acute lethality caused by nitrogen toxicity is uncommon in Canada. This is due, in part, to restrictions imposed upon municipal authorities and industries to ensure that wastewater discharges are appropriately treated and diluted to safeguard aquatic ecosystems and humans that use these waters. The chronic effects of elevated concentrations of ammonia, nitrate, nitrite, and nitrogen dioxide are less well understood. Because the symptoms of chronic nitrate toxicity may mimic or be masked by other environmental changes, the extent of chronic nitrogen toxicity in the environment is generally not known. Excess nutrients from various anthropogenic sources (municipal wastewater effluent, agriculture, etc.) have led to occurrences of severe toxicity in Canada. For example, groundwater contamination, fish kills, outbreaks of shellfish toxicity, cyanobacteria-related livestock deaths, and declines in amphibian populations have all been related to excessive nutrients. We will present Canadian case studies where nutrient loading in the environment is problematic. This work is part of an ongoing project by the Government of Canada to quantitatively and qualitatively determine the impacts of nutrients from various sources (i.e., atmospheric, agriculture/aquaculture, municipal, and industrial) on the Canadian environment.

Identifying Persistent, Bioaccumulative and Toxic Substances in Canadian Commerce: How Difficult can it be? R. Breton, R. Chénier, C. Gagnon, P. Harris, D. MacDonald and R. Sutcliffe. Environment Canada, Commercial Chemicals Evaluation Branch, Hull, QC.

Abstract
Under the revised *Canadian Environmental Protection Act*, Environment Canada and Health Canada must "categorize" and "screen" 23,000 substances that are considered to be in Canadian commerce and therefore listed on the Domestic Substances List (DSL) to determine whether they are "toxic" as defined in the Act. This paper focuses on the environmental aspect of this project only. The environmental categorization identifies persistent and/or bioaccumulative and toxic (PBT) substances on the DSL. The criteria used to categorize these substances are those set out in the Toxic Substances Management Policy. An Advisory Group and several working groups have been set up to provide recommendations on how to resolve scientific and technical issues that emerge from implementation of this project. A pilot project has been initiated which will identify 100 substances representative of several chemical classes of concern.

Introduction
Concerns relating to persistent bioaccumulative substances have been expressed for the past few decades. Concerted actions to deal with these have been seen in particular over the past ten years. Canada has participated in activities at several different levels. The federal government adopted the Toxic Substances Management Policy in 1995 (Government of Canada 1995a). The policy promotes a preventative precautionary approach to substance management in all federal initiatives, and calls for the virtual elimination of persistent bioaccumulative substances that satisfy specific criteria (Track 1 substances). Recently, 12 substances were identified as satisfying criteria for Track 1 (Department of Environment 1998). The policy recognizes the importance of on-going domestic and international efforts to deal with these substances. These include actions taken with the provinces, such as the Canada-Ontario Accord, the Great Lakes Action Plan and the Saint-Laurent Vision 2000, which all target persistent bioaccumulative substances. More recently, the Canadian Council of Ministers of the Environment adopted a Policy for the Management of Toxic Substances that calls for cooperative work in the prioritization, assessment and management of toxic substances, with particular focus on actions for persistent bioaccumulative substances. Canada has also been working with the US as reflected in the Canada-US Binational Strategy on Toxic Substances. Continental efforts include the Sound Management of Chemicals initiatives pursuant to the North American Free-Trade Agreement that identifies substances of mutual concern that warrant continental management actions. Regional initiatives include the UN Economic Council for Europe protocol for POPs under the Long Range Transboundary Air Pollutants Convention. Most recently, Canada is actively participating in the development of a globally binding convention on POPs though the UN Environmental Protection Program.

Federal powers to support Canadian actions in these initiatives are derived from various federal legislation. Key among these is the *Canadian Environmental Protection Act* (CEPA), that provides for the assessment and management of substances that can be released into the Canadian environment. The CEPA provides for the protection of the environment and of the health of Canadians from toxic substances and other pollutants. The legislation was enacted in 1988. It provided for actions on toxics that focused on the assessment, management and clean up of existing pollution. It has had a positive impact on how Canada deals with environmental challenges and has made possible many environmental achievements.

Provisions in CEPA call for a review of the Act after 5 yrs. The review that has been on-going for the past few years has focused on new expectations of Canadians and developments in environmental law and scientific knowledge since 1988. The new CEPA has been modernized and strengthened by making pollution prevention the cornerstone of national efforts to reduce toxic substances in the environment. It shifts the focus from cleaning up environmental problems to preventing them in the first place. The Act received Royal Assent on September 14, 1999, and should be promulgated in early 2000. The new legislation requires the government to assess more substances more quickly, and set firm deadlines for action to control toxic substances. Several new mandates have been introduced in the Act which proposes a more efficient process of identifying, screening, assessing and managing toxic substances.

Mandate under CEPA

Although the new CEPA is still not promulgated, Environment Canada has initiated the implementation of some of the new initiatives. One of these initiatives involves the identification of persistent, bioaccumulative and toxic substances (PBTs) that are in Canadian commerce. It requires the Minister of the Environment and the Minister of Health to "categorize" and then "screen" substances listed on the Domestic Substances List (DSL) to determine whether they are "toxic" as defined in the Act. Under the Act, a substance is "toxic" if it is entering or may enter the environment in a quantity or concentration or under conditions: [a having or that may have an immediate or long-term harmful effect on the environment; [b] constituting or that may constitute a danger to the environment on which human life depends; or [c] constituting or that may constitute a danger in Canada to human life or health.

The DSL includes substances that were, between January 1, 1984, and December 31, 1986, in Canadian commerce, used for manufacturing purposes, or manufactured in or imported into Canada in a quantity of 100 kg or more in any calendar year. The List has been amended from time to time and currently contains approximately 23 000 substances. Types of substances on the DSL include simple organic chemicals, pigments, organometallic compounds, surfactants, polymers, metal elements, metal salts and other inorganic substances, as well as substances that are of "Unknown or Variable Composition, complex reaction products, or Biological materials" (referred to as UVCBs) (Fig. 1).

Fig. 2 outlines the various uses for which these substances were notified in 1986. Substances could be included under any of 50 different functional use groups, including an "other" category (6%). The largest represented use group is "other industrial uses," which is a combination of over 30 use patterns such as absorbents, abrasives, analytical reagents, catalysts, flame retardants, fuel additives and many more. The three single largest uses were fragrances, perfumes, deodorizers and flavouring agents (14%), polymers (11%), and colorants such as pigments, stains, dyes and inks (10%).

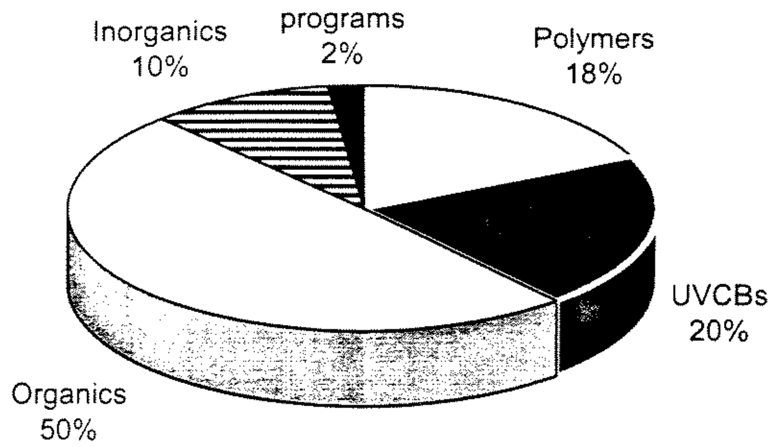


Fig. 1. Types of substances on the Domestic Substances List

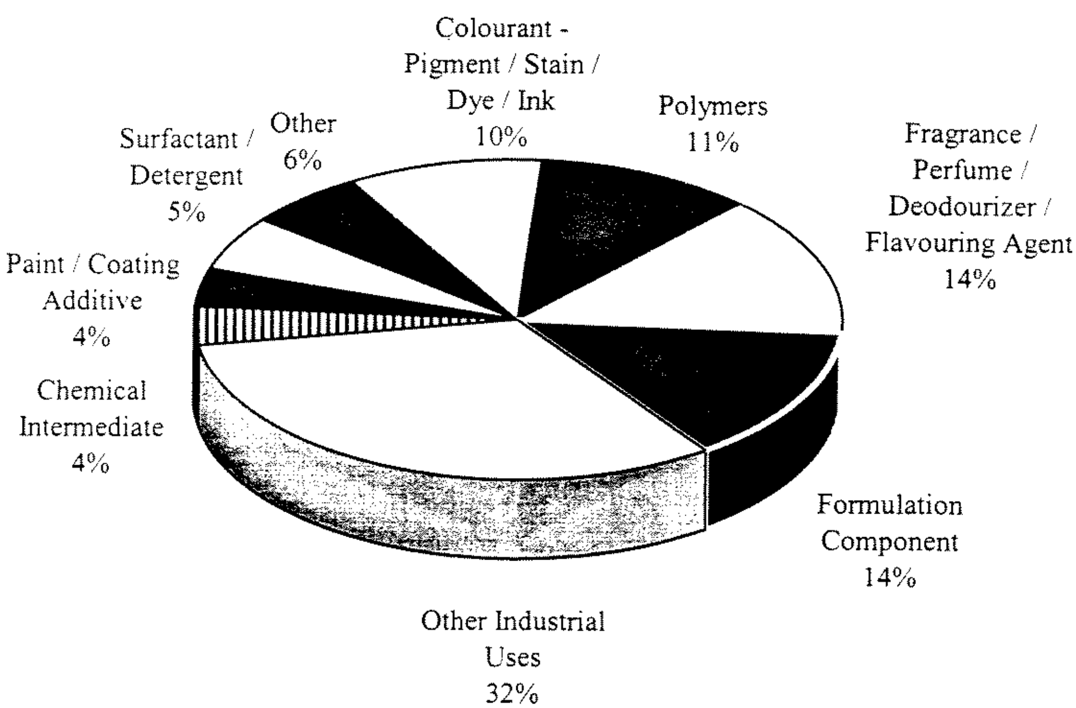


Fig. 2. Reported use patterns for substances on the Domestic Substances List in 1986

Many different industrial sectors notified these substances (Fig. 3). The largest ones were the organic chemicals sector (12%), the pigment, dye and printing ink sector (10%) and the paint and coating sector (9%). The "other" sector represents over 30 combined industry sectors, including adhesive and sealant production, construction materials, mining, metal and non-metal and organometallic chemicals.

Since most of the substances on the DSL have not undergone any environmental or human health assessment, the new CEPA provides for the systematic assessment of substances on the DSL that are to be done in two phases. The initial phase, referred to as the categorization of substances, identifies substances that will proceed to the second phase, a screening level risk assessment (Fig. 4). The environmental categorization of substances on the DSL requires the Minister of the Environment to assess the substances on the basis of their inherent toxicity, persistence, and bioaccumulation potential. The criteria for persistence (P) and bioaccumulation (B) are to be derived from those in the Toxic Substances Management Policy (TSMP) and, as stated in CEPA, will be included in regulations that are currently being developed. The definition and interpretation of inherent toxicity for this exercise is currently under development as are the approaches for categorizing substances based on inherent toxicity.

Criteria for persistence and bioaccumulation set out in the TSMP were selected by an expert panel of scientists, based on knowledge of the properties that are most characteristic of persistent organic pollutants (Government of Canada 1995b). The criteria were chosen to identify substances that are likely to be of greatest concern with regards to persistence and bioaccumulation. Persistence is based on a consideration of all environmental media. A substance is considered persistent if its transformation half-life satisfies the criterion in any one medium as identified in Table I. A substance is considered as bioaccumulative if any of the bioaccumulation criteria in Table I are satisfied.

As identified in the new CEPA, all substances on the DSL must be categorized within 7 years after the Bill receives Royal Assent (September 14, 1999). If a substance does not meet the criteria, then no further action is required for this substance under this categorization and screening exercise. When a substance is categorized as satisfying the criteria for persistence, bioaccumulation and inherent toxicity, then a screening level risk assessment (SLRA) is required (Fig. 4). The SLRA involves a more in-depth analysis of a substance to determine whether the substance is "toxic" or capable of becoming "toxic" as defined in CEPA. A SLRA results in one of the following outcomes: [1] no further action is taken at this time, if the SLRA indicates that the substance does not pose a risk to the environment or human health; [2] the substance is added to the CEPA Priority Substances List in order to assess more comprehensively the possible risks associated with the release of the substance, if the substance is not already on the Priority Substances List but the SLRA indicates possible concerns; or [3] it is recommended that the substance be added to the List of Toxic Substances in Schedule 1 of CEPA, if the SLRA indicates clear concerns, whether these are associated or not with the persistence or bioaccumulation properties of the substance; substances on Schedule 1 can be considered for regulatory or other controls.

As mandated under the new CEPA, where, based on a screening level risk assessment, a substance may have a long-term harmful effect on the environment because it is persistent, bioaccumulative, inherently toxic and predominantly anthropogenic, then the substance will be added to the List of Toxic Substances. If, in addition, the substance is not a naturally occurring

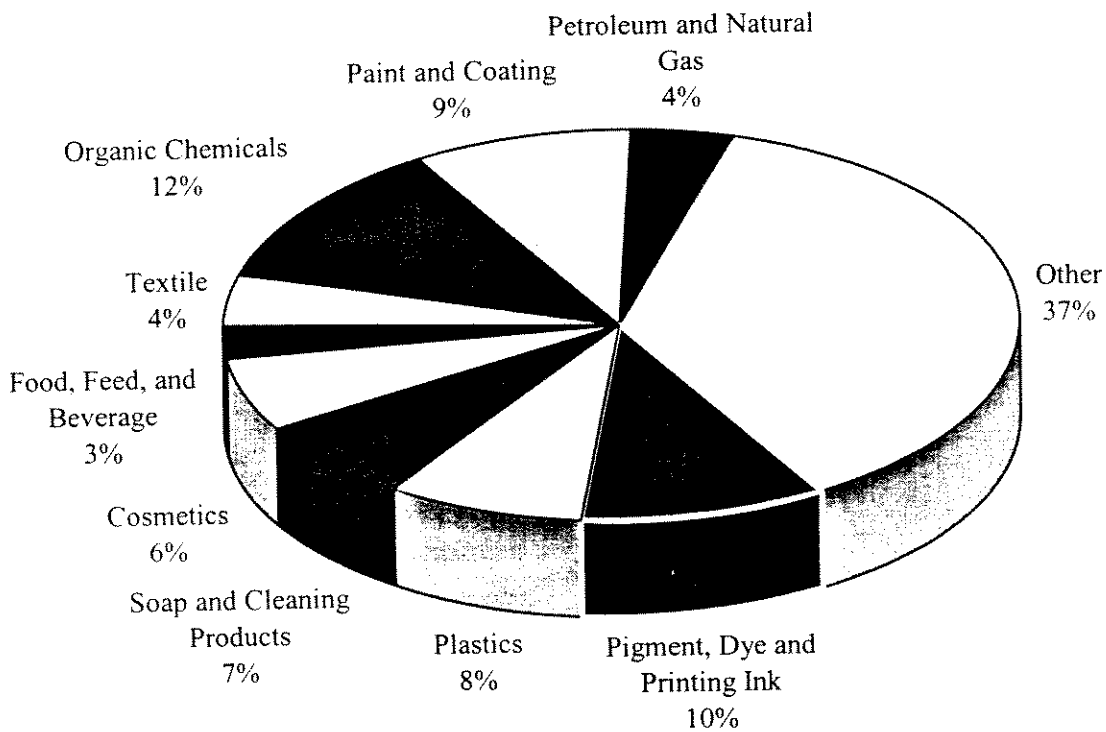


Fig. 3. Reported industrial sectors for substances on the Domestic Substances List in 1986

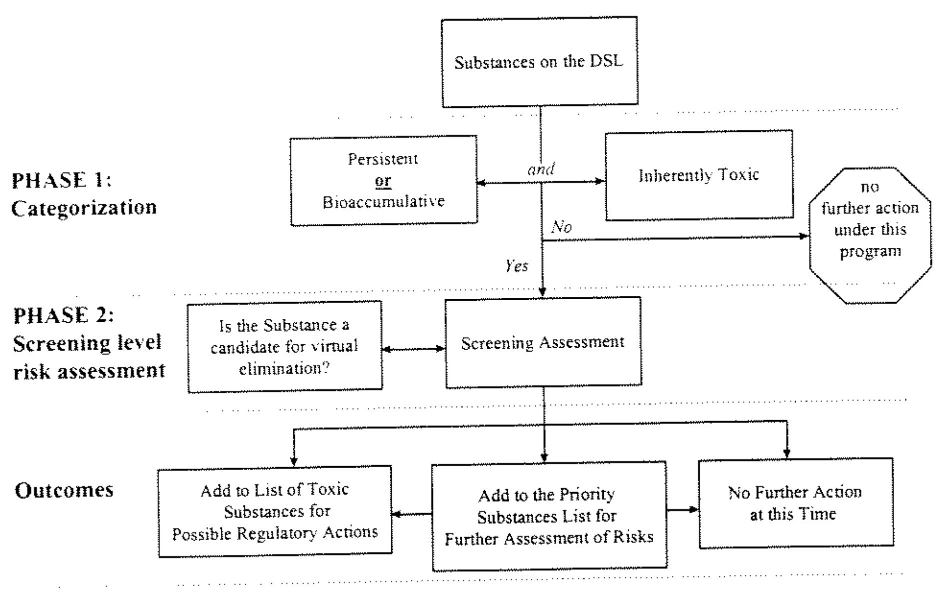


Fig. 4. Environmental categorization and screening of the Domestic Substances List

Table 1. Criteria for persistence and bioaccumulation

Persistence[a]		Bioaccumulation[c]
Medium	Half-life	BAF[d] ≥5,000
Air	≥ 2 days[b]	or
Water	≥ 6 months	BCF[e] ≥ 5,000
Sediment	≥ 1 year	or
Soil	≥ 6 months	LogKow ≥ 5

[a]A substance is considered persistent when the criterion is met in any one medium.
[b]A substance may be considered as persistent in air if it is shown to be subject to atmospheric transport to remote regions such as the Arctic.
[c]Bioaccumulation Factors (BAF) are preferred over Bioconcentration Factors (BCF); in the absence of BAF or BCF data, the octanol-water partition coefficient (log K_{ow}) may be used.
[d]Bioaccumulation factor means the ratio of the concentration of a substance in an organism to the concentration in water, based on uptake directly from the surrounding environment and food.
[e]Bioconcentration factor means the ratio of the concentration of a substance in an organism to the concentration in water, based only on uptake directly from the surrounding environment.

inorganic substance or a naturally occurring radionuclide, then the substance will be proposed for virtual elimination under CEPA. In addition to certain substances that have undergone screening level risk assessments, substances that have not been identified as being of concern through the categorizing and screening exercise can nonetheless be assessed. The Priority Substances Assessment Program is mandated under CEPA. Under this mandate, the Minister of the Environment and the Minister of Health develop a list of substances, known as the Priority Substances List, that should be given priority for assessment to determine whether they are "toxic" as defined under CEPA. Substances that are assessed as "toxic" may be placed on the List of Toxic Substances (Schedule 1 of the Act), and be considered for possible risk management measures such as regulations, guidelines, or codes of practice to control any aspect of their life cycle, from the research and development stage through manufacture, use, storage, transport and ultimate disposal.

Issues, Challenges and Approach

Data on persistence, bioaccumulation and inherent toxicity needed to conduct the categorization are only available for a small number of the substances on the DSL. Tools such as Quantitative Structure Activity Relationships (QSARs) used to predict these data are not available for many of the classes or types of substances on the DSL (e.g., pigments, polymers, ionic surfactants). Some of the challenges regarding modeling include the estimation of media-specific half-lives, the estimation of the bioaccumulation potential for some classes of substances (e.g., pigments), the estimation of toxicity of many classes of substances (e.g., organometallic substances, surfactants), and the use and development of fate and exposure models for the screening level risk assessments. In addition, the nature of some of these substances, such as polymers, UVBCs, and metal elements and metal salts poses a challenge when applying the criteria for the categorization. One of the challenges, considering the many hundreds of inorganic substances on the DSL, will involve determining the inherent toxicity of such substances and taking into account concepts such as bioavailability and transformation.

The development of approaches and methods for conducting a SLRA is currently underway. A risk based approach will be used and will consist of four major parts: characterization of entry, exposure, effects and risk. The objective of the entry characterization will be to determine the uses, types of releases and amounts of the substance entering the Canadian environment. These data will then be used as input to the characterization of exposure which is most likely to involve modeling. The objective of the exposure characterization will be to determine estimated exposure concentrations to biota. Characterization of effects will first consider experimental data. However, in most cases, due to the lack of experimental data, QSARs will be used to estimate toxicity. The final part, risk characterization, involves determining the likelihood of adverse effects to biota. This will be carried out by combining the results of the characterization of entry, exposure and effects. As stated in the new CEPA, when conducting and interpreting the results of a SLRA, a weight of evidence approach and the precautionary principle will be applied.

An Advisory Group that consist of experts from government, industry, environmental organizations and consultant groups, have been set up to provide an expert resource to Environment Canada for identifying and assisting in resolving issues of a scientific, technical and process nature that emerge from implementation of the project. The group will assist in the preparation of technical guidance documents and identify approaches to conduct the categorization and screening assessments. The Advisory Group will also assist in establishing technical Working Groups as required to carry out specific tasks.
Two Working Groups and a workshop are currently being set up. The first Working Group will be responsible for developing an approach to fragment or divide the DSL into manageable groups of similar substances. The second Working Group will be responsible for recommending a practical approach for categorizing inorganic substances against the criteria for persistence, bioaccumulation and inherent toxicity. In addition, a workshop on QSARs is being organized by Environment Canada. The workshop will bring together about 30 international experts on estimating persistence, bioaccumulation and toxicity. The objectives of the workshop include recommending which QSAR to use for the categorization of different types of substances, identifying alternatives to QSARs for substances for which QSARs are not available and recommending research for data gaps identified. The workshop is scheduled for November 11-12, 1999, in Philadelphia. A workshop report will be available in the Spring of 2000.

Environment Canada has initiated a pilot project which will identify 100 substances representative of several chemical classes of concern with regards to persistence, bioaccumulation and inherent toxicity. This will involve the evaluation of a list of over 7,000 organic substances. The categorization of these substances will be completed by the winter of 1999-2000. Environment Canada will be carrying out screening-level risks assessments as substances are identified that meet the categorization criteria. The screening assessments for substances identified in the pilot project are scheduled to be completed by March 2001. For each subsequent year, screening assessments will be carried out for a next group of substances identified as being of concern.

The methods, approaches and results of the categorization and screening level risk assessments will be collated in technical guidance documents and reports. In addition, a database containing the technical data used to conduct the categorization and screening assessments is currently being developed. Decisions and documents will be publicly available. A web site for this project is currently being developed and will be linked to the Commercial Chemicals Evaluation Branch web site which can be found at http://www.ec.gc.ca/cceb1/.

Conclusion

One of the new initiatives under the proposed revised *Canadian Environmental Protection Act* states that Environment Canada must "categorize" and "screen" substances that are listed on the Domestic Substances List (DSL) to determine whether they are "toxic" as defined in the Act. The environmental categorization identifies persistent and/or bioaccumulative and toxic (PBT) substances on the DSL. The criteria used to categorize these substances are those set out in the Toxic Substances Management Policy. Because of the difficulty in determining the persistence, bioaccumulation and inherent toxicity of the various classes of substances on the DSL and in conducting screening level risk assessments on these substances, Environment Canada has set up several mechanisms including an Advisory Group and several working groups in order to provide guidance on how to resolve scientific and technical issues that emerge from implementation of this project. A pilot project has been initiated which will identify 100 substances representative of several chemical classes of concern.

Because many of the data on persistence, bioaccumulation and inherent toxicity are only available for a small number of the substances on the DSL, tools such as Quantitative Structure Activity Relationships (QSARs) will be used to predict these data. However, current models are not sophisticated enough to predict reliable estimates for all classes of substances on the DSL. Some of the challenges regarding modeling include the estimation of media-specific half-lives, the estimation of the bioaccumulation potential for some classes of substances (e.g., pigments), the estimation of toxicity of many classes of substances (e.g., organometallic substances, surfactants). In addition, the nature of some of these substances, such as polymers, UVCBs, and metal elements and metal salts poses a challenge when applying the criteria for the categorization. One of the topics of discussion for the workshop on QSARs organized for November 1999 will include recommendations for research and development of models for data gaps that are identified.

References

Government of Canada. 1995a. Toxic Substances Management Policy; June 1995.

Government of Canada. 1995b. Toxic Substances Management Policy, Persistence and Bioaccumulation Criteria.

Department of the Environment. 1998. Notice concerning the confirmation of "Track 1 substance" status for aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, PCBs, polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, and toxaphene, as determined by assessment of these substances against the Track 1 criteria of the Toxic Substances Management Policy. Canada Gazette, Part I, 4 July 1998, p. 1568-1569.

A Revised Approach to Applying Uncertainty Factors in the Ecological Effects Assessment of New Substances in Canada. M. Bonnell[1], A. Atkinson[2], G. Hammond[2] and E. Postlethwaite[2].
[1]Bonnell Environmental Consulting, Ottawa, ON; [2]Environment Canada, Hull, QC.

The ecological effects assessment of new substances in Canada requires the use of uncertainty (safety) factors to estimate toxicity threshold concentrations of chemicals or polymers notified under the New Substances Regulations of the *Canadian Environmental Protection Act* (CEPA). A study was conducted to "update" the traditional USEPA-based approach currently used to assess new substances in Canada in order to provide a more transparent, data-derived method to determining uncertainty factors. The revised method estimates a residual level of uncertainty based on the calculation of component "certainty factors" that account for pivotal study quality, inter- and intra-specific variation, acute to chronic extrapolation, severe effects to no effects extrapolation and laboratory to field extrapolation. The results of five new substance case studies, which compared the revised approach with the USEPA-based approach, and conclusions and recommendations of the study are also presented.

LIST OF AUTHORS/LISTE DES AUTEURS

Author and page

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

BEST PLATFORM PRESENTATION

Does the Potency of Polycyclic Aromatic Hydrocarbons (PAH) for Inducing CYP1A in Juvenile Trout (*Oncorhynchus mykiss*) Predict Dioxin-like Toxicity in Early Life Stages?

Sonya Billiard
Department of Biology, Queen's University, Kingston, ON

BEST POSTER PRESENTATION

A Dialysis Mini-peeper for Measuring Pore Water Metal Concentrations in Laboratory Sediment Toxicity and Bioavailability Tests

Lorne Doig
Toxicology Centre, University of Saskatchewan, Saskatoon, SK

LIST OF REGISTRANTS/LISTE DES PARTICIPANTS

L Name	F Name	Association	Location	Telephone	Email
Adams	Marshall	Oak Ridge National Lab.	Oak Ridge, TN		
Aidun	Bijan	Alberta Environment	Edmonton, AB	780-427-0636	bijan.aidun@gov.ab.ca
Andersen	Anee-Marie	Alberta Environment	Edmonton, AB	780-427-2980	aanderso@env.gov.ab.ca
Anderson	Donald	Environment Canada	Ottawa, ON	819 953 1538	donald.andersen@ec.gc.ca
Andrews	Daniel	Western Resource Solutions	Calgary, AB	403-640-7075	dabond@sprynet.com
Antoniolli	Wendy	Environment Canada	Edmonton, AB	780-435-7301	WendyAntoniolli@ec.gc.ca
Arsenault	Darryl	EBA Engineering Consultants	Kelowna, BC	250-862-4832	darsenault@eba.ca
Atkinson	Glenn F.	Atkinson Statistical	Toronto, ON	416-694-6254	gfatstat@aol.com
Backus	Sean	Environment Canada, CCIW	Burlington, ON		sean.backus@cciw.ca
Baddaloo	Earle	Government of Nunavut	Iqaluit, Nunavut	867-979-5119	ebaddaloo@gov.nu.ca
Bailey	Howard	EVS Environment Consultants	N. Vancouver, BC	604-986-4331	hbailey@evs.bc.ca
Bartlett	Adrienne	University of Waterloo	Burlington, ON	905-336-4405	adrienne.bartlett@cciw.ca
Basu	Nil	University of British Columbia	Vancouver, BC	604-228-4849	nilbasu@interchange.ubc.ca
Battigelli	Jeff	University of Alberta	Edmonton, AB		jbattige@ualberta.ca
Bayer	Barb	Enviro-Test Laboratories	Winnipeg, MB	204-945-2280	bbayer@wpg.envirotest.com
Bennett	Kirsty	Simon Fraser University	Burnaby, BC	604-291-5985	kirsty@sfu.ca
Bertram	Barbara	Wilfrid Laurier University	Waterloo, ON	519-725-4767	bert0550@mach1.wlu.ca
Bézaire Dussault	Ève	University of Waterloo	Waterloo, ON	519-888-4567	ebezaire@sciborg.uwaterloo.ca
Billiard	Sonya	Queen's University	Kingston, ON	513-533-6000	billiard@biology.queensu.ca
Birkholz	Deib	Enviro-Test Laboratories	Edmonton, AB	780-413-5205	deib@envirotest.com
Blazer	Vicky	U.S.GS, VSGS Fish Health Lab	Kearneysin, WV	304-724-4434	vicki_blazer@usgs.goc
Bollo-Kamara	Arthur	Alberta Environment	Edmonton, AB	780-427-0636	
Borgmann	Anne	Environment Canada	Downsview, ON	416-739-5939	Anne.Borgmann@ec.gc.ca
Borgmann	Uwe	Environment Canada, CCIW	Burlington, ON	905-336-6280	uwe.borgmann@cciw.ca
Braekevelt	Eric	University of Alberta	Edmonton, AB	780-434-5161	ericb@connect.ab.ca
Brandt	Kent	Transalta	Wabamun, AB	780-892-5525	kent_brandt@transalta.com
Breton	Roger	Environment Canada	Hull, QC	819-953-1650	roger.breton@ec.gc.ca
Brown	Stephen	Queen's University	Kingston, ON	613-533-2655	browns@chem.queensu.ca
Brown	Maureen	Maxxam Analytics Inc.	Edmonton, AB	780-468-3545	mbrown@mail.edm.maxxam.ca
Brownlee	Brian	Environment Canada, CCIW	Burlington, ON	905-336-4706	Brian.Brownlee@cciw.ca
Bruno	Joy	Environment Canada	N. Vancouver, BC	604-924-2518	Joy.Bruno@ec.gc.ca
Buchanan	Randy	Buchanan Environmental Ltd.	Fredericton, NB	506-450-4463	buchenv@fundy.net
Buday	Craig	Environment Canada	N. Vancouver, BC	604-924-2514	Craig.Buday@ec.gc.ca
Burnison	B. Kent	Environment Canada, CCIW	Burlington, ON	905-336-4407	kent.burnison@cciw.ca
Burridge	Les	Dept. of Fisheries and Oceans	St. Andrews, NB	506-529-5903	burridgel@mar.dfo-mpo.gc.ca
Cabral	Carlson	University of Waterloo	Waterloo, ON	519-746-7512	cpcabral@sciborg.uwaterloo.ca

Callin	Erv	Enviro-Test Laboratories	Edmonton, AB	780-413-5244	
Carr	Annie	Canisius College, Biology	Buffalo, NY	716-888-2555	dehn@canisius.edu
Casey	Richard	Alberta Environment	Edmonton, AB	780-427-1583	Richard.Casey@gov.ab.ca
Caux	Pierre-Yves	Environnement Canada	Ottawa, ON	819-953-0602	pierre-yves.caux@ec.gc.ca
Chan	Teresa	Queen's University	Kingston, ON	613-533-6000	c/o billiard@biology.queensu.ca
Chapman	Peter	EVS Environment Consultants	N. Vancouver, BC	604-986-4331	pchapman@attglobal.net
Chouinard	Alain	Government of Nunavut	Arviat, Nunavut	867-857-2828	achouinard@gov.nu.ca
Christie	Carolyn	HydroQual Laboratories Ltd.	Calgary, AB	403-253-7121	hydroqua@cadvision.com
Chung	Susanna	Weyerhaeuser Canada	Grande Prairie, AB	780-539-8131	susanna.chung@weyerhaeuser.com
Ciborowski	Jan	University of Windsor	Windsor, ON	519-253-3000	cibor@uwindsor.ca
Clendenan	Melinda	EBA Engineering Consultants Ltd.	Edmonton, AB	780-451-2121	mclendenan@eba.ca
Cline	Patricia	Golder Associates Inc.	Gainesville, FL	352-336-5600	patricia_cline@golder.com
Cloutier	Isabelle	Abitibi-Consolidated Inc.	Montreal, QC	514-394-3700	isabelle_cloutier@abicon.com
Clyde	Geordie	Jacques Whitford Environmental	St. John's, NF		
Coady	Kim	Environment Canada	Mount Pearl, NF	709-722-4087	kim,coady@ec.gc.ca
Colavecchia	Maria	Queen's University	Kingston, ON	905-607-6053	Pfuts@msn.com
Collier	Tracy K.	NOAA, , NW Fish. Sci. Center	Seattle, WA	206-860-3312	tracy.k.collier@noaa.gov
Compton	Rhona	Cantox Environmental Inc.	Calgary, AB	403-237-0275	rcompton@cantoxenvironmental.com
Constable	Miles	Environment Canada	Edmonton, AB	780-951-8732	miles.constable@ec.gc.ca
Cooke	Sandra	AB Agri, Food & Rural Devlop.	Edmonton, AB	780-427-3397	Sandra.Cooke@agric.gov.ab.ca
Cooley	Megan	North/South Consultants	Winnipeg, MB	204-284-3366	nscons@nscons.mb.ca
Craig	Gordon	G.R. Craig & Associates Inc.	Bolton, ON	905-859-3701	grcraig@ibm.net
Culp	Robin	Canisius College, Biology	Buffalo, NY	716-888-2555	dehn@canisius.edu
Davies	Martin	Hatfield Consultants Ltd.	W. Vancouver, BC	604-926-3261	mdavies@hatfieldgroup.com
Dayabhai	Dharmesh	Web Spinners Canada Inc.	Toronto, ON	416-740-1862	dharmesh@sympatico.ca
C.de Rosemond	Simone	University of Saskachewan	Saskatoon, SK	306-966-7441	crocquet@sask.esask.ca
Dehn	Paula	Canisius College, Biology	Buffalo, NY	716-888-2555	dehn@canisius.edu
Dixon	D. George	University of Waterloo	Waterloo, ON	519-885-1211	dgdixon@sciborg.uwaterloo.ca
Dobson	Evan	Hatfield Consultants Ltd.	W. Vancouver, BC	604-926-3261	edobson@hatfieldgroup.com
Doe	Kenneth	Environment Canada	Moncton, NB	506-851-3486	ken.doe@ec.gc.ca
Doig	Lorne	University of Saskachewan	Saskatoon, SK	306-966-4882	ldoig@dlcwest.com
Donahue	Bill				
Duncan	Bill F.A.	Cominco Ltd.	Trail, BC	250-364-4336	bduncan@trail.cominco.com
Dutton	Mike	Cantox Environmental Inc.	Calgary, AB	403-237-0275	
Easton	Michael	International EcoGen Inc.	N. Vancouver, BC	604-986-2400	michael_easton@intl-ecogen.com
Eickhoff	Curtis	BC Research	Vancouver, BC	604-224-4331	ceickhoff@bcresearch.com
Elliott	Garth	Environment Canada	Edmonton, AB	780-435-7242	garth.elliott@ec.gc.ca

Ernst	Bill	Environment Canada	Dartmouth, NS	902-426-5048	bill.ernst@ec.gc.ca
Evans	J. Catherine	University of Waterloo	Waterloo, ON	519-888-4567	jcevans@sciborg.uwaterloo.ca
Evereklian	Gary	Azur Environmental	Carlsbad, CA	760-438-8282	evereklian@aol.com
Fairbairn	Margaret	Environment Canada		780-951-8750	
Fennell	Michelle	Environment Canada	N. Vancouver, BC	604-924-2516	michelle.fennell@ec.gc.ca
Filiatrault	Dionne	Nunavut Water Board	Gjoa Haven, Nunavut	867-360-6338	dionne@polarnet.ca
Firth	Barry	Weyerhaeuser Canada	Tacoma, WA	253-924-6946	
Fitzgerald	Dean	University of Windsor	Windsor, ON	519-253-3000	fitzge8@uwindsor.ca
Fraikin	Chris	Golder Associates Ltd.	Calgary, AB	403-299-4666	cfraikin@golder.com
Gabugi	K. M.	LAM Geotechnics Ltd.	Hong Kong, China	852-2897-3282	gabuji@lamconstruct.com.hk
Gagnon	Christian	Environment Canada	Ottawa, ON	819-997-1617	christian.gagnon@ec.gc.ca
Gaudet	Connie	Environment Canada	Ottawa, ON	819-953-3199	connie.gaudet@ec.gc.ca
Gaudet	Irene	Alberta Research Council	Vegreville, AB	780-632-8468	irene@arc.ab.ca
George	Tara	University of Sask	Saskatoon, SK	306-966-7441	geroge@sask.usak.ca
Gibbons	Wade	Golder Associates Ltd.	Calgary, AB	403-299-5651	Wade_Gibbons@Golder.com
Gilron	Guy	ESG International	Guelph, ON	519-836-6050	ggilron@esg.net
Glozier	Nancy	Environment Canada	Saskatoon, SK	306-975-6057	Nancy.Glozier@ec.gc.ca
Goeres	Michael	CCME			
Gong	Ping	National Research Council	Montreal, QC	514-496-6279	ping.gong@nrc.ca
Goudey	J. Stephen	HydroQual Laboratories Ltd.	Calgary, AB	403-253-7121	hydroqua@cadvision.com
Gould	Rachel	University of Waterloo	Waterloo, ON	519-888-4567	rlgould@uwaterloo.ca
Govenlock	Mary	Alberta Newsprint Company	WhiteCourt, AB	780-778-7055	maryg@nt1.altanewsprint.ca
Grasman	Keith	Wright State University	Dayton, OH	937-775-2106	keith.grasman@wright.edu
Greene	Joe	U.S. EPA, Environ. Res.	Corvallis, OR	541-754-4773	greenejo@proaxis.com
Grundy	Stephen	Royal Roads University	Victoria, BC	250-391-2579	steve.grundy@royalroads.ca
Guay	Isabelle	MENV	Quebec City, QC	418-521-3820	isabelle.guay@mef.gouv.qc.ca
Hackbarth	Tracy	Queen's University	Edmonton, AB	780-435-5235	6th2@qlink.queensu.ca
Hagen	Mike	Environment Canada	N. Vancouver, BC	604-666-6544	mike.hagen@ec.gc.ca
Hall	Ken	Govt. of Northwest Territories	Yellowknife, NT	867-873-7651	
Halliwell	Douglas	Environment Canada	Yellowknife, NT	867-669-4741	doug.halliwell@ec.gc.ca
Hamilton	Janice	HydroQual Laboratories Ltd.	Calgary, AB	403-253-7121	hydroqua@cadvision.com
Hamoutene	Dounia	Memorial University	St. John's, NF	709-737-8536	dhamoute@morgan.ucs.mun.ca
Haya	Kats	Dept. of Fisheries and Oceans	St. Andrews, NB	506-529-5916	hayak@mar.dfo-mpo.gc.ca
Hebben	Thorsten	University of Alberta	Edmonton, AB	780-492-5615	thebben@ualberta.ca
Hedley	Kathleen	Environment Canada	Hull, QC	819-953-1553	kathleen.hedley@ec.gc.ca
Heelan Powell	Brenda		Calgary, AB	403-263-1323	
Heinze-Milne	Sigfried	CFB Green, Environ. Office	Greenwood, NS	902-765-7793	nstn.3052@fo.nstn.ca

Heland-Powell	Brenda	Alberta Justice			
Hellou	Jocelyne	Dept. of Fisheries and Oceans	Dartmouth, NS	902-426-7451	hellouj@mar.cfo-mpo.gc.ca
Himbeault	Kevin	Conor Pacific	Saskatoon, SK	306-242-4442	kevin.himbeault@conorpac.com
Himmelright	Justin		Vancouver, BC	604-685-0545	jmimmelright@knightpiesold.com
Hodson	Peter	Queen's University	Kingston, ON	613-533-6129	hodsonp@biology.queensu.ca
Iafrancesco	Maria	HydroQual Laboratories	Calgary, AB	403-253-7121	
Irving	Elaine	Environment Canada	Saskatoon, SK	306-975-4655	elaine.irving@ec.gc.ca
Jackson	Francis	Indian and Northern Affairs Can.	Yellowknife, NT	867-669-2666	JacksonF@INAC.GC.CA
Jardine	Janet J	Dept. of Fisheries and Oceans	Burlington, ON	905-336-4465	jardinej@dfo-mpo.gc.ca
Jenkins	Lynda	Alberta Justice	Edmonton, AB	780-422-9728	lynda.jenkins@just.gov.ab.ca
Jensen	Fern	Environment Canada	Edmonton, AB	780-951-8868	fern.jensen@ec.gc.ca
Juergensen	Lars	Environment Canada	Ottawa, ON	819-997-4070	juergensen.lars@ec.gc.ca
Kardash	Marianne	DIAND	Yellowknife, NT	867-669-2756	kardashm@inac.gc.ca
Kendall	Joe	AB Agric, Food & Rural Develop.	Edmonton, AB	403-427-2270	
Kent	Robert	Environment Canada	Ottawa, ON	819-953-1554	robert.vent@ec.gc.ca
Khan	Haseen	Newfoundland Environment	St. John's, NF	709-729-2535	hkhan@mail.gov.nf.ca
Kierstead	Ted		Point Edward, ON	519-337-6484	kierstt@ebtech.net
Kim	Kay	Environment Canada	Dartmouth, NS	902-426-8564	kay.kim2@ec.gc.ca
king	Thomas	Dept. of Fisheries and Oceans		902-426-4172	KingT@mar.dfo-mpo.gc.ca
Kinnee	Karen	UBC/BC Research	Vancouver, BC	604-224-4331	kkinnee@bcresearch.com
Kirby	Susanna	Azur Enviromental	Carlsbad, CA		skirby@azurenv.com
Knafla	Anthony	Equilibrium Environmental Inc.	Calgary, AB	403-286-7706	tknafla@home.com
Knight	Barry	Maxxam	Edmonton, AB	780-468-3529	bknight@maxxam.ca
Kohli	Mohan	Environment Canada, CCIW	Burlington, ON	905-336-4470	mohan.kohli@cciw.ca
Koning	Wendell	Alberta Environment	Calgary, AB	403-297-8267	wendell.koning@gov.ab.ca
Koop	Cindy	First Nations		780-483-8601	
Koper	Nicky	University of Alberta			
Kruper	Nancy	Environment Canada	Edmonton, AB	780-435-7307	nancy.kruper@ec.gc.ca
Kung	Irene	HydroQual Laboratories Ltd.	Calgary, AB	403-253-7121	hydroqua@cadvision.com
Labrie	Jacques	Alcan Primary Metal Group	Jonquiere, QC	418-699-6585	jacques_labrie@alcan.com
Laird	Scott	Enviro-Test Laboratories	Edmonton, AB	708-413-5205	
Langer	Julia	World Wildlife Fund Canada	Toronto, ON	416-489-4567	jlanger@wwfcanada.org
Larose	Celine	Capital Regional District	Victoria, BC	250-360-3144	clarose@crd.bc.ca
Latonis	Graham	Bovar Waste Management	Sawn Hills, AB	980-333-4197	
Latreille	Alain	Environnement Canada	Montreal, QC	514-496-6854	alain.latreille@ec.gc.ca
LeClair	Doreen	Alberta Environment	Edmonton, AB	780-427-2712	
Lee	Robert C.	Golder Associates Ltd.	Calgary, AB	403-299-4634	rlee@golder.com

Liber	Karsten	University of Saskachewan	Saskatoon, SK	306-966-7441	karsten.lober@usask.ca
lickacz	Bob	Alpha Laboratory Services	Edmonton, AB	403-439-9100	
Lindeman	Dorothy	Environment Canada	Saskatoon, SK		
Lopez	Bobby	Web Spinners Canada Inc.	Toronto, ON	416-740-1862	bobby.lopez@sympatico.ca
Lopez-Gastey	José	Montreal Urban Community	Montreal, QC	514-280-4288	jose.lopez.gastey@cum.qc.ca
Lowell	Richard	Environment Canada, NWRI	Saskatoon, SK	306-975-6303	Rick.Lowell@ec.gc.ca
Luebke	Robert	U.S. Environ. Protection Agency	Res. Tri. Park, NC	919-541-3672	luebke.robert@epa.gov
Lumb	Ashok	Environment Canada, EMAN	Burlington, ON	905-336-4413	ashok.lumb@cciw.ca
MacDonald	Gord	Diavik	Calgary, AB	403-261-6100	gord.macdonald@diavik.com
MacDonald	Adrian	Environment Canada	Dartmouth, NS	902-426-8305	adrian.macdonald@ec.gc.ca
Macdonald	Ron	Greater Vancouver Regional Dist.	Burnaby, BC	604-451-6632	ron.macdonald@gvrd.bc.ca
MacGregor	Don	Environment Canada	Ottawa, ON	613-990-9540	MacGregor.Don@etc.ec.gc.ca
MacKinnon	Mike	Syncrude Canada Ltd.	Edmonton, AB	780-970-6890	mackinnon.michael@syncrude.com
Madajczuk	Anna	NOVA Chemicals Corporation	Sarnia, ON	519-481-3518	madajca@novachem.com
Mah-Paulson	May	O'Connor Associates Environ. Inc.	Calgary, AB	403-294-4256	may-mahpaulson@oconnor-associates.com
Martel	Pierre	PAPRICAN	Pointe Claire, QC	514-630-4100	pmartel@paprican.ca
Martin	Robert	Nova Chemicals	Red Deer, AB	403-314-7534	martinbw@novachem.com
Masters	Andrew	Maxxam Analytics Inc.	Edmonton, AB	780-468-3522	amasters@mail.edm.maxxam.ca
McCarthy	Lynda	Ryerson Polytechnic University	Toronto, ON	416-979-5000	L3mccart@acs.ryerson.ca
McCoy	Greg	Millar Western	Whitehouse, AB	780-778-2036	
McGreer	Eric	ERM Environmental Consulting	Vancouver, BC	604-732-6841	mcgreer@bc.sympatico.ca
McKernan	Mike	Tetres Consultants Inc.	Winnipeg, MB	204-942-2505	mmckernan@tetres.ca
McLean	Richard	Irving Pulp & Paper	Saint John, NB	506-653-5001	mclean.richard@irvingpulp.com
McMaster	Mark	Environment Canada, CCIW	Burlington, ON	905-319-6906	Mark.McMaster@cciw.ca
McRory	Susan	Alberta Justice	Edmonton, AB	780-422-9727	susan.mcrory@just.gov.ab.ca
Mehta	Ram D.	PBR Laboratories Inc.	Edmonton, AB	780-450-3957	pbr@oanet.com
Meinelt	Thomas	Inst. Freshw. Biology/Inland Fish.	Berlin, Germany	+3064190560	meinelt@igb-berlin.de
Miskimmin	Brenda	Limnos Freshwater Consultants	Vernon, BC	250-549-3589	limnos@bctel.ca
Mitchell	Ian	O'Connor Associates Environ. Inc.	Calgary, AB	403-294-4257	ian-mitchell@oconnor-associates.com
Mitchell	Patricia	Alberta Environment	Edmonton, AB	780-427-2981	pmitchel@env.gov.ab.ca
Moen	Andrea	Fraser Milner Barr. & Solicitors	Edmonton, AB	780-423-7100	andrea.moen@frasermilner.com
Moody	Mary	Saskatchewan Research Council	Saskatoon, SK	306-933-5469	moody@src.sk.ca
Moore	Dan	Alberta Newsprint Company	WhiteCourt, AB	780-778-7028	danm@nt1.altanewsprint.ca
Moran	Tim S.	Pollutech Enviroquatics Ltd.	Point Edward, ON	519-339-8787	tmoran@pollutech.com
Moul	David	Pacific Environ. Science Centre	N. Vancouver, BC	604-924-2517	David.Moul@ec.gc.ca
Mroz	Rita	Environment Canada	Dartmouth, NS	902-426-9405	rita.moriz@ec.gc.ca
Mucklow	Laura	Golder Associates Ltd.	Calgary, AB	403-299-4662	laura_mucklow@golder.com

Munro	Scott	Lambton Industrial Society	Sarnia, ON	519-332-2010	lis@ebtech.net
Munteanu	Nina	G3 Consulting Ltd.	Richmond, BC	604-231-9856	nina@g3consulting.com
Murphy	Sean	Komex International Ltd.	Edmonton, AB	780-496-9055	smurphy@edmonton.komex.com
Murphy	Clair	PEI Dept of Technol and Environ.	Charlottetown, PE	902-368-5036	ccmurphy@gov.pe.ca
Nason	Ted	Alberta Environment	Edmonton, AB		
Nelson	Andrew	Northwest Power Corp.	Hay River, NT	867-874-5248	anelson@ntpc.com
Niimi	Arthur	Dept. of Fisheries and Oceans	Burlington, ON	905-336-4868	niimia@dfo-mpo.gc.ca
Norwood	Warren	Environment Canada, CCIW	Burlington, ON	905-336-4694	Warren.Norwood@CCIW.ca
Noton	Leigh	Alberta Environment	Edmonton, AB	780-427-2899	Leigh.Noton@gov.ab.ca
Novak	Lesley	ESG International	Guelph, ON	509-836-6050	lnovak@esg.net
Odense	Remi	BC Ministry of Environment	Smithers, BC	250-847-7224	Remi.Odense@gems7.gov.bc.ca
Oishi	Courtney	Komex International Ltd.	Calgary, AB	403-247-0200	coishi@calgary.komex.com
Paine	Michael	Paine, Ledge and Associates	N. Vancouver, BC	604-924-8126	mdpaine_pla@bc.sympatico.ca
Palace	Vince	Dept. of Fisheries and Oceans	Winnipeg, MB	204-983-5004	palacev@dfo-mpo.gc.ca
Pammer	Cindy	Golder Associates Ltd.	Calgary, AB	403-216-8992	cpammer@golder.com
Paquin	Emery	Gov. of Northwest Territories		867-873-7651	
Parker	Roy	Environment Canada	Fredericton, NB	506-452-3234	roy.parker@ec.gc.ca
Parrott	Joanne	Environment Canada, CCIW	Burlington, ON	905-336-4551	joanne.parrott@caw.ca
Pauls	Ronald	Syncrude	Fort McMurray, AB	403-790-8382	pauls.ron@syncrude.com
Payne	Jerry	Dept. of Fisheries and Oceans	St. John's, NF	709-772-2089	
Pedlar	Roberta	University of Manitoba/DFO	Winnipeg, MB	204-222-1753	rmpedlar@home.com
Pennell	Lisa	Environment Canada	Edmonton, AB	780-435-7389	Lisa.Pennell@ec.gc.ca
Penney	Kathy	Jacques Whitford Environment	St. John's, NF	709-576-1458	kpenny@jacqueswhitford.com
Perron	Francine	Environment Canada	Montreal, QC	514-283-0195	francine.perron@ec.gc.ca
Peters	Lisa	University of Waterloo	Waterloo, ON	519-888-4567	lepeters@sciborg.uwaterloo.ca
Pickard	Janet	B.C. Research Inc.	Vancouver, BC	604-224-4331	jpickard@bcresearch.com
Pierre	Martel	Paprican	Pointe Claire, QC	514-630-4100	pmartel@paprican.ca
Playle	Richard	Wilfrid Laurier University	Waterloo, ON	519-884-0710	rplayle@wlu.ca
Porebski	Linda	Environment Canada	Hull, QC	819-953-4341	Linda.Porebski@ec.gc.ca
Portt	Cam	C. Portt & Associates	Guelph, ON	519-824-8227	cportt@sentex.net
Postlethwaite	Emma	Environment Canada	Ottawa, ON	613-739-0750	emma.postlethwaite@ec.gc.ca
Ptashynski	Melanie	Dept. of Fisheries and Oceans	Winnipeg, MB	204-983-5005	ptashynskim@dfo-mpo.gc.ca
Purdy	John	Novartis Crop Protection Can.	Guelph, ON	519-837-5323	john.purdy@cp.Novartis.com
Ratliffe	Will	EBA Engineering	Edmonton, AB	780-451-2130	wratliffe@eba.ca
Raymond	Beverley	Environment Canada	Vancouver, BC	604-664-4053	beverly.raymond@ec.gc.ca
Renoux	Agnis	National Research Council	Montreal, QC	514-496-2663	agnes.renoux@nrc.ca
Ribey	Sandra	Environment Canada	Hull, QC	819-953-3456	sandra.ribey@ec.gc.ca

Riehm	Derek	Teck Corporation	Vancouver, BC	604-687-1117	driehm@teckcorp.ca
Roberts	Elizabeth	(ESR)2 Consulting	Nepean, ON	819-997-1029	roberts.elizabeth@ec.gc.ca
Robins	Geneva	Komex International Ltd.	Calgary, AB	403-247-0200	grobins@calgary.komex.com
Robinson	Rick	Golder Associates Ltd.	Calgary, AB	403-299-5666	rrobinson@golder.com
Rodrigue	Danielle	Environment Canada	Gloucester, ON	613-990-9544	rodrigue.danielle@etc.ec.gc.ca
Roe	Susan	Environment Canada	Ottawa, ON	819-994-8405	susan.roe@ec.gc.ca
Rogers	Robert	Toxcon HSRC. Inc.	Edmonton, AB	780-435-9028	bobr@oanet.com
Rogers	Vince	University of Saskachewan	Saskatoon, SK	306-966-7441	vvr121@mail.usask.ca
Romanko	Wade	Environment Canada	Yellowknife, NT	867-669-4736	wade.romanko@ec.gc.ca
Rosaasen	Arden	Cogema Resources Inc.	Saskatoon, SK	306-244-2554	arosaasen@sk.sympatico.ca
Rosemond	Simone de	University of Saskachewan	Saskatoon, SK	306-966-7441	crocquet@sask.usask.ca
Ross	Lauren		Qualicum Beach, BC	250-752-4443	laurenross@canada.com
Ross	Peter	Institute of Ocean Sciences	Sidney, BC	250-363-6806	rosspe@pac.dfo-mpo.gc.ca
Roy	Robert	IML, Pêches et Océans	Mont-Joli, QC	418-775-0647	royro@dfo-mpo.gc.ca
Ryan	Michael	Queens University	Kingston, ON	204-955-9494	madoilspot@netscape.net
Saffran	Karen	Alberta Environment	Edmonton, AB	780-427-2662	ksaffran@env.gov.ab.ca
Salahub	Bob	Norwest Labs	Edmonton, AB	780-438-5522	bobs@norwestlabs.com
Salazar	Sandra	Applied Biomonitoring	Kirkland, WA	425-823-3905	msalazar@cnw.com
Salazar	Michael	Applied Biomonitoring	Kirkland, WA	425-823-3905	msalazar@cnw.com
Schneider	Uwe	Environment Canada	Ottawa, ON	819-953-8599	uwe.schneider@ec.gc.ca
Schroeder	Julie	Beak International Inc.	Brampton, ON	905-794-2325	jschroeder@beak.com
Schwartz	Mellissa	Wilfrid Laurier University	Waterloo, ON	519-884-0710	schw3230@mach1.wlu.ca
Scott	Jennifer	HydroQual Laboratories Ltd.	Calgary, AB	403-253-7121	
Scroggins	Rick	Environment Canada	Gloucester, ON	613-990-8569	scroggins.richard@etc.ec.gc.ca
Sentis	Randy	Cominco Ltd.	Trail, BC	250-364-4238	rsentis@trail.cominco.com
Serben	Kerrie	Canadian Wildlife Service	Saskatoon, SK	306-975-4791	kerrie.serben@ec.gc.ca
Sevigny	James	Komex International Ltd.	Calgary, AB	403-247-0200	jsevigny@calgary.komex.com
Shalagan	Jennifer	O'Connor Associates Environ. Inc.	Calgary, AB	403-294-4252	
Shelast	Bob	Stantec Consulting Ltd.	Calgary, AB	403-716-8134	bshelast@stantec.com
Sherry	Jim	Environment Canada, CCIW	Burlington, ON	905-336-4813	jim.sherry@cciw.ca
Shipton	Jeff	Millar Western	WhiteCourt, AB	780-778-2036	
Siwik	Paula	University of Alberta	Alix, AB	403-747-2192	rbps@telusplanet.net
Skinner	Neil	Conor Pacific	Edmonton, AB	780-450-0909	neil.skinner@conorpac.com
Smith	Sherri	Environment Canada	Ottawa, ON	819-953-3082	smith.sherri@ec.gc.ca
Smits	Judit	University of Saskatchewan	Saskatoon, SK	306-966-7445	smits@skyway.usask.ca
Sobey	Kirk G.	Golder Associates Ltd.	Saskatoon, SK	306-665-7989	kirk_sobey@golder.com
Soehnlein	Stephanie	Canisius College, Biology	Buffalo, NY	716-888-2555	dehn@canisius.edu

Somers	Jim	Standards Council of Canada	Ottawa, ON	613-238-3222	jsomers@scc.ca
Sosiak	Al	Alberta Environment	Calgary, AB	403-297-5921	
Spafford	Mark	Alberta-Pacific Industries Inc.	Boyle, AB	780-525-8400	spaffoma@alpac.ca
Spankie	James	Northwood Inc.	Prince George, BC	250-962-3747	james_spankie@northwood.ca
Spry	Douglas	Ontario MOE	Etobicoke, ON	416-235-5799	sprydo@ene.gov.on.ca
Steenkamer	Alka	Environment Canada	Ottawa, ON	613-990-9647	Steenkamer.Alka@etc.ec.gc.ca
Stefanik	Melanie	Beak International Inc.	Brampton, ON	905-794-2325	mstefanik@beak.com
Stein	Warren	Komex International Ltd.	Edmonton, AB	780-496-9055	wstein@edmonton.komex.com
Stephenson	Gladys	ESG International	Guelph, ON	519-836-6050	gstephenson@esg.net
Sunahara	Geoffrey	National Research Council	Montreal, QC	514-496-8030	geoffrey.sunahara@nrc.ca
Sutcliffe	Roger	Environment Canada	Hull, QC	819-994-2381	roger.sutcliffe@ec.gc.ca
Swansburg	Erin	University of Windsor	Windsor, ON	519-253-4232	taylo1x@server.uwindsor.ca
Swanson	Stella	Golder Associates Ltd.	Calgary, AB	403-299-4606	sswanson@golder.com
Tabash	Samir	Queen's University	Kingston, ON	613-533-6000	tabashs@chem.queensu.ca
Tank	Suzanne	University of Alberta	Edmonton, AB	780-492-1292	suztank@ualberta.ca
Taylor	Darrell	N.S. Dept. of the Environment	Halifax, NS	902-424-2570	taylordl@gov.ns.ca
Taylor	Kenneth	Environment Canada	Hull, QC	819-953-3976	Ken.Taylor@ec.gc.ca
Tetreault	Gerald	University of Waterloo/ EC	Burlington, ON	905-336-6269	Gerald.Tetreault@odin.cciw.ca
Thellen	Claude	Min. de l'environ. du Quebec	Sainte-Foy, QC	418-643-8225	claude.thellen@mef.gouv.qc.ca
Thomas	Peter	Covance Laboratories Inc	Madison, WI	608-245-7007	peter.thomas@covance.com
Thomas	Gregory P.	G3 Consulting Ltd.	Richmond, BC	604-231-9856	gthomas@g3consulting.com
Thompson	Gregg	Conor Pacific	Edmonton, AB	780-450-0909	
Thompson	Gary	Westbank Frist Nation	Kelowna, BC	250-707-3332	
Tindal	Miles	Komex International Ltd.	Calgary, AB	403-247-0200	mtindal@calgary.komex.com
Touart	Leslie	U. S. EPA	Washington, DC	703-305-6134	touart.les@epa.gov
Tremblay	Alain	Hydro Quebec	Montreal, QC	514-840-3000	tremblay.alain@hydro.qc.ca
Tremblay	Louis	Landcare Research	Lincoln, New Zealand	64 3 325 6700	TremblayL@landcare.cri.nz
Trudel	Lise	Environment Canada	Hull, QC	819-953-1571	lise.trudel@ec.gc.ca
Valupadas	Prasad	Alberta Environment	Edmonton, AB	780-427-0636	prasad.valupadas@gov.ab.ca
van Aggelen	Graham	Environment Canada	N. Vancouver, BC	604-924-2513	graham.vanaggelen@ec.gc.ca
Van Coillie	Raymond	University of Quebec	Sainte-Foy, QC	418-656-0098	
Vanden Heuvel	Mike	N.Z. Forest Research Institute	Rotorua, New Zealan	64-7-347-5899	mike.vandenheuvel@forestresearch.co.nz
Verbeek	Allen	Conor Pacific	Edmonton, AB	780-450-0909	allen.verbeek@conorpac.com
Vos	Joseph G.	Nat.Inst.Public Health & Environ.	Bilthoven, Netherland	31-30-2742075	j.vos@rivm.nl
Wasylenchuk	Elaine	Alberta Environment	Edmonton, AB	780-427-0636	
Watson	Glen	INCO Ltd., Environment	Copper Cliff	705-682-8231	gwatson@inco.com
Wells	Peter	Environment Canada	Dartmouth, NS	902-426-1426	peter.wells@ecgc.ca

Westlake	Gary	Ontario Min. of Environment	Etobicoke, ON	416-235-5797	westlaga@ene.gov.on.ca
Whelly	Mark	University of Windsor	Windsor, ON	519-253-3000	whelly@uwindsor.ca
White Sobey	Sue	University of Saskachewan	Saskatoon, SK	306-966-8841	whitess@sask.usask.ca
Whitley	Gerry	DIAND Water Resources	Whitehorse, YK	867-667-3217	whitleyg@inac.gc.ca
Whittle	D. Michael	Dept. of Fisheries and Oceans	Burlington, ON	905-336-4565	whittlem@dfo-mpo.gc.ca
Whyte	Jeff	USGS, Col. Environ Res. Center	Columbia, MO	573-875-5399	Jeff_Whyte@usgs.gov
Williamson	Dwight	Manitoba Environment	Winnipeg, MB	204-945-7030	dwilliamso@gov.mb.ca
Wilson	Guy	Weyerhaeuser Canada	Grande Prairie, AB	780-539-8129	guy.wilson@wyerhaeuser.com
Wilson	Jeffrey	HydroQual Laboratories Ltd.	Calgary, AB	403-253-7121	
Wirgin	Isaac	NYU School of Medicine	Tuxedo, NY	914-731-3548	wirgin@env.med.nyu.edu
Witteman	John	BHP Diamonds Inc.	Yellowknife, NT	867-880-2232	witteman.john.jp@bhp.com.au
Wolanski	Alina	Luscar Ltd.	Edmonton, AB	780-420-5848	alina_wolanski@luscar.com
Young	Leslie	Hatfield Consultants Ltd.	W. Vancouver, BC	604-926-3261	lyoung@hatfieldgroup.com
Young	Iris	Dept of Nat. Defence, QETE	Hull, QC	819-994-1681	i.young@issc.debbs.ndhq.dnd.ca
Zajdlik	Barry	Zajdlik & Associates	Rockwood, ON	519 856-9440	bzajdlik@sentex.net
Zelikoff	Judy	NYU School of Medicine	New York, NY	914-731-3528	judyz@env.med.nyu.edu
Zemanek	Mike	Alberta Environment	Edmonton, AB	780-427-0636	
Zhao	Yuhui	Enviro-Test Laboratories	Edmonton, AB	780-413-5290	henryz@envirotest.com
Ziervogel	Herb	EBA Engineering Consultants Ltd.	Edmonton, AB	780-451-2130	hziervogel@eba.ca
Zosimadis	Mike	Web Spinners Canada Inc.	Toronto, ON	416-740-1862	mike@ws.ca
Zurawell	Ron	University of Alberta	Edmonton, AB	780-492-1261	rzurawel@gpu.srv.ualberta.ca

WORKSHOP PROCEEDINGS/COMPTE RENDUS D'ATELIER

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).

Comptes rendus du 25e colloque annuel de toxicologie aquatique: 18-21 octobre 1998, Québec, Québec. Éditeurs: R. Van Coillie, R. Chassé, C. Julien, L. Martel, C. Thellen et A.J. Niimi, M.D. Treissman and A.J. Niimi. Can. Tech. Rep. Fish. Aquat. Sci. 2260: 134 p.

Proceedings of the 24th Annual Aquatic Toxicity Workshop: October 19-22, 1997, Niagara Falls, Ontario. Edited by A.J. Niimi, G.L. Parrott and D.G. Spry. Can. Tech. Rep. Fish. Aquat. Sci. 2192: 135 p.

Proceedings of the 23rd Annual Aquatic Toxicity Workshop: October 7-9, 1996, Calgary, Alberta. Edited by J.S. Goudey, S.M. Swanson, M.D. Treissman and A.J. Niimi. Can. Tech. Rep. Fish. Aquat. Sci. 2144: 196 p.

Proceedings of the 22nd Annual Aquatic Toxicity Workshop: October 2-4, 1995, St. Andrews, New Brunswick. Edited by K. Haya and A.J. Niimi. Can. Tech. Rep. Fish. Aquat. Sci. 2093: 159 p.

Proceedings of the 21st Annual Aquatic Toxicity Workshop: October 3-5, 1994, Sarnia, Ontario. Edited by G.F. Westlake, J.L. Parrott and A.J. Niimi. Can. Tech. Rep. Fish. Aquat. Sci. 2050: 179 p.

Proceedings of the 20th Annual Toxicity Aquatic Workshop: October 17-21, 1993, Quebec City, Quebec. Edited by R. Van Coillie, Y. Roy, Y. Bois, P.G.C. Campbell, P. Lundahl, L. Martel, M. Michaud, P. Riebel and C. Thellen. Can. Tech. Rep. Fish. Aquat. Sci. 1989: 331 p.

Proceedings of the 19th Annual Aquatic Toxicity Aquatic Workshop: October 4-7, 1992, Edmonton, Alberta. Edited by E.G. Baddaloo, S. Ramamoorthy and J.W. Moore. Can. Tech. Rep. Fish. Aquat. Sci. 1942: 489 p.

Proceedings of the 18th Annual Aquatic Toxicity Workshop: September 30-October 3, 1991, Ottawa, Ontario. Edited by A.J. Niimi and M.C. Taylor. Can. Tech. Rep. Fish. Aquat. Sci. 1863: 381 p.

Proceedings of the 17th Annual Aquatic Toxicity Workshop: November 5-7, 1990, Vancouver, British Columbia. Edited by P. Chapman, F. Bishay, E. Power, K. Hall, L. Harding, D. McLeay, M. Nassichuck and W. Knapp. Can. Tech. Rep. Fish. Aquat. Sci. 1774: 1213 p.

Proceedings of the 15th Annual Aquatic Toxicity Workshop: November 28-30, 1988, Montreal, Quebec. Edited by R. Van Coillie, A.J. Niimi, A. Champoux and G. Joubert. Can. Tech. Rep. Fish. Aquat. Sci. 1714: 244 p.

Proceedings of the 14th Annual Aquatic Toxicity Workshop: November 2-4, 1987, Toronto, Ontario. Edited by A.J. Niimi and K.R. Solomon. Can. Tech. Rep. Fish. Aquat. Sci. 1607: 201 p.

Proceedings of the 13th Annual Aquatic Toxicity Workshop: November 12-14, 1986, Moncton, New Brunswick. Edited by J.S.S. Lakshminarayana. Can. Tech. Rep. Fish. Aquat. Sci. 1575: 178 p.

Proceedings of the 12th Annual Aquatic Toxicity Workshop: November 5-8, 1985, Thunder Bay, Ontario. Edited by G. Ozburn. Can. Tech. Rep. Fish. Aquat. Sci. 1462: 229 p.

Proceedings of the 11th Annual Aquatic Toxicity Workshop: November 13-15, 1984, Vancouver, British Columbia. Edited by G. Geen and K.L. Woodward. Can. Tech. Rep. Fish. Aquat. Sci. 1480: 330 p.

Proceedings of the 10th Annual Aquatic Toxicity Workshop: November 7-10, 1983, Halifax, Nova Scotia. Edited by P.G. Wells and R.F. Addison. Can. Tech. Rep. Fish. Aquat. Sci. 1368: 475 p.

Proceedings of the 9th Annual Aquatic Toxicity Workshop: November 1-5, 1982, Edmonton, Alberta. Edited by W.C. Mckay. Can. Tech. Rep. Fish. Aquat. Sci. 1163: 243 p.

Proceedings of the 8th Annual Aquatic Toxicity Workshop: November 2-4, 1981, Guelph, Ontario. Edited by N.K. Kaushik and K.R. Solomon. Can. Tech. Rep. Fish. Aquat. Sci. 1151: 255 p.

Proceedings of the 7th Annual Aquatic Toxicity Workshop: November 5-7, 1980, Montreal, Quebec. Edited by N. Bermingham, C. Blaise, P. Couture, B. Hummel, G. Joubert and M. Speyer. Can. Tech. Rep. Fish. Aquat. Sci. 990: 519 p.

Proceedings of the 6th Annual Aquatic Toxicity Workshop: November 6-7, 1979, Winnipeg, Manitoba. Edited by J.F. Klaverkamp, S.L. Leonhard and K.E. Marshall. Can. Tech. Rep. Fish. Aquat. Sci. 975: 291 p.

Proceedings of the 5th Annual Aquatic Toxicity Workshop: November 7-9, 1978, Hamilton, Ontario. Edited by P.T.S. Wong, P.V. Hodson, A.J. Niimi, V. Cairns and U. Borgmann. Fish. Mar. Ser. Tech. Rep. 862: 342 p.

Proceedings of the 4th Annual Aquatic Toxicity Workshop: November 8-10, 1977, Vancouver, British Columbia. Edited by J.C. Davis, G.L Greer and I.K. Burtwell. Fish. Mar. Ser. Tech. Rep. 818: 211 p.

Proceedings of the 3rd Annual Aquatic Toxicity Workshop Held in Halifax, Nova Scotia, November 2-3, 1976. Edited by W.R. Parker, E. Pessah, P.G. Wells and G.F. Westlake. Environment Canada, Surveillance Rep. EPS-5-AR-77-1.

Proceedings of the 2nd Annual Aquatic Toxicity Workshop, November 4-5, 1975, Rexdale, Ontario. Edited by G.R. Craig. Ontario Ministry of the Environment.

Compendium of Aquatic Toxicity Studies in Canada. 1974. Unpublished Report, Freshwater Institute, Winnipeg, Manitoba. 39 p. + appendices.