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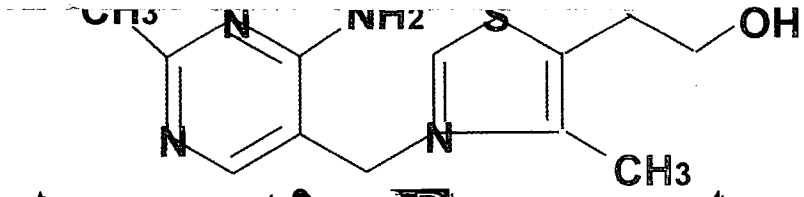
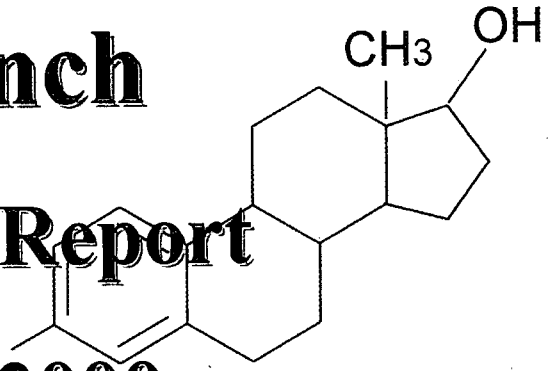


Aquatic Ecosystem Protection Branch



Annual Report

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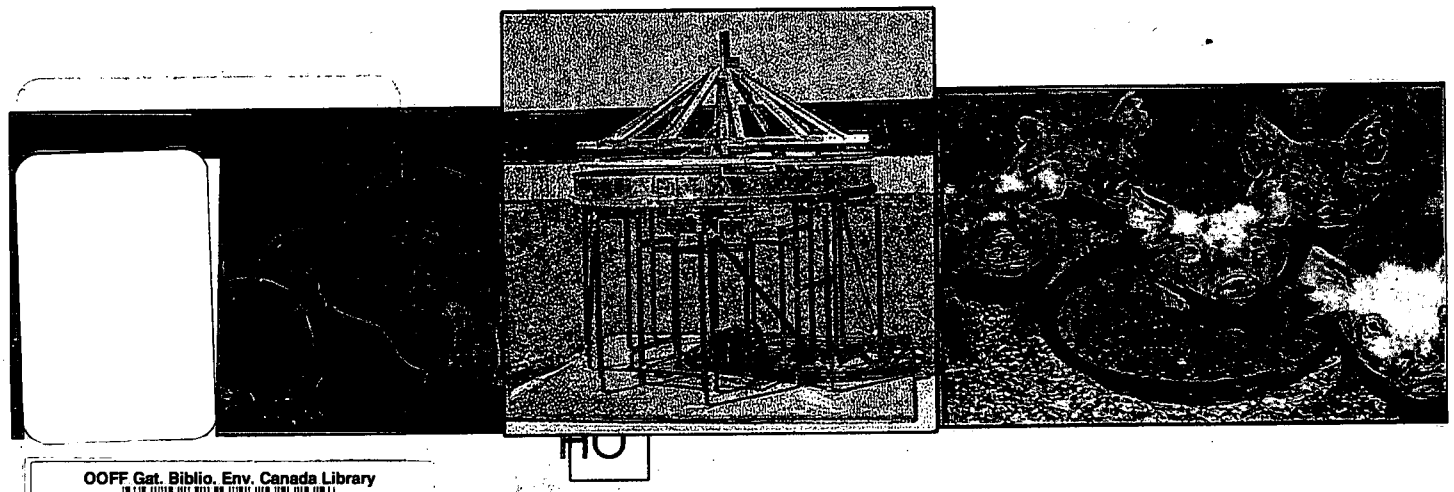

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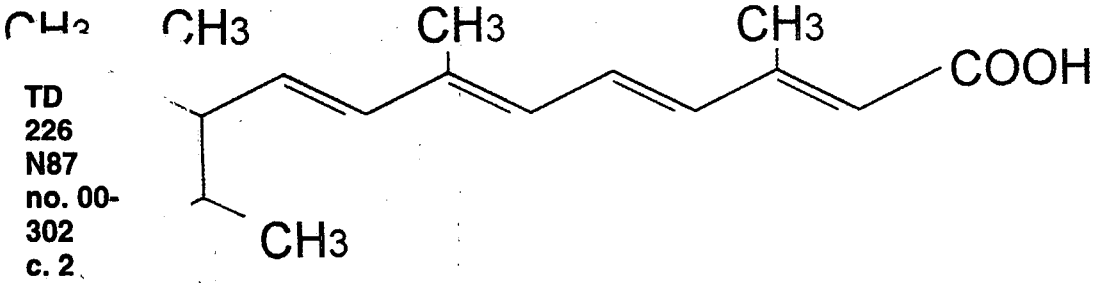

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Non Point Sources of Pollution Project; Fiscal year 1999-2000
Project Chief: Jiri Marsalek

The Project provides important scientific information on sources, transport, effects and control of non-point source pollution impacting Canadian aquatic ecosystems, and thereby supports the Clean Environment and Nature components of the Departmental Management Framework. This information is used by Environment Canada in the management and delivery of regional ecosystem programs, and also to make decisions under the Toxic Substances Management Policy and the Canadian Environmental Protection Act. The Project accomplishes its goals by researching processes governing the generation, transport and prevention of non-point source pollution, and also by providing related services to others.

Current activities include the assessment of wet-weather pollution from urban and agricultural lands; transport of pollutants from such sources; interactions of pollutants with sediment, flocs and biofilm; the modelling of pollution source/effect relationships; and mitigation of non-point source pollution by best management practices. All current studies include collaborative research with others.

Project Chief	J. Marsalek
Study Leaders	I.G. Droppo
	A. El-Shaarawi
	B.G. Krishnappan
	L. Lau
	J.R. Lawrence
	G.G. Leppard
	H.F.Y. Ng
	W.B. Taylor

Research Technologists	W. Christian
	J. Heidt
	C. Jaskot
	B. Near
	R. Stephens
	G. Swerhone

Sources diffuses de pollution, année financière 1999-2000
Responsable : Jiri Marsalek

L'équipe de ce projet recueille d'importants renseignements scientifiques sur les sources, le transport, les effets et la réduction de la pollution diffuse dans les écosystèmes aquatiques du Canada. Le projet contribue ainsi aux volets de l'assainissement de l'environnement et de la nature du cadre de gestion ministériel. Environnement Canada applique ces connaissances dans la mise en oeuvre et à l'administration de programmes régionaux visant les écosystèmes, ainsi que dans les prises de décisions effectuées dans le cadre de la Politique de gestion des substances toxiques et de la *Loi canadienne sur la protection de l'environnement*. Pour atteindre les objectifs du projet, on s'efforce d'élucider les mécanismes régissant la production, le transport et la prévention de la pollution diffuse, et on fournit des services connexes à d'autres intervenants.

Au nombre des activités en cours, on compte l'évaluation de la pollution issue des terres urbaines et agricoles par temps de pluie, l'étude du transport des polluants à partir de ces sources, l'établissement des interactions entre ces polluants et les sédiments, les flocs et les films biologiques, la modélisation des rapports entre les source et les effets des polluants, et l'atténuation de la pollution diffuse par l'application des meilleures pratiques de gestion. Toutes les études en cours comprennent une collaboration avec d'autres chercheurs.

Responsable	J. Marsalek
Chefs d'études	I.G. Droppo
	A. El-Shaarawi
	B.G. Krishnappan
	L. Lau
	J.R. Lawrence
	G.G. Leppard
	H.F.Y. Ng
	W.B. Taylor
Technologues de recherche	W. Christian
	J. Heidt
	C. Jaskot
	B. Near
	R. Stephens
	G. Swerhone

Microorganisms and Contaminants

Studies were conducted on the effects of nutrients and contaminants on development of river microbial biofilms, and the role(s) played by these microbial systems in the fate of contaminants and of genetically modified bacteria. Suitable model systems were developed and tested for the cultivation of river biofilm communities for experimental applications

Rotating Annular Reactors Study Leader: **John R. Lawrence**	Contribution to ***Clean Environment; Toxics Result*** *of EC's management framework*

Two types of rotating annular reactors were evaluated for their ability to cultivate river biofilms. One, a commercially available system with a rotating inner solid cylinder and polycarbonate slides in the outer fixed cylinder--cost 15-20K--while the second, a custom designed system with polycarbonate slides positioned on a machined, rotating inner cylinder cost $100-200.00. Experimental comparison of these biofilms was carried out using confocal laser scanning microscopy techniques including, fluorescent nucleic acid staining, fluor conjugated lectins and autofluorescence imaging. The results indicated that the reactors were similar in terms of biofilm development pattern, thickness, bacterial biomass, and exopolymer production. Significant differences were, however, found in terms of photosynthetic biomass, with the glass bodied non-commercial reactor providing more favourable conditions for algal growth than the opaque polycarbonate outer cylinder of the commercial reactor. The study indicated that a simple inexpensive reactor produced river biofilms similar to those obtained using a commercial system, but at substantially lower cost. The availability of inexpensive annular reactors will promote replicated studies of river biofilm development at a mesocosm scale and permit thorough assessment of the impacts of stressors on riverine microbial communities.

Effects of microorganisms on the fate of toxics in river biofilms Study Leader: **John R. Lawrence**	Contribution to ***Clean Environment; Toxics Result*** *of EC's management framework*

Mesocosm experiments were conducted to show the impact of specific microorganisms on the fate of toxics in river biofilms. A novel bacterial colony morphology was observed in river water which had been cultivated in rotating annular reactors and viewed using epi-fluorescence and confocal laser scanning microscopy (CLSM). This microcolony type had a complex exopolymeric coating. Studies using CLSM techniques, SEM and x-ray microprobe analyses showed that the exopolymers were involved in binding heavy metals such as nickel and cadmium. Binding was at 5-10X the concentration of other biofilm materials. These results show the potential importance of the presence and abundance of specific populations in sequestration and sorption of contaminants within

naturally occurring river biofilms, and the importance of evaluating community composition when showing the potential role of microbial biofilms in the fate of contaminants.

Degradation of Diclofop-methyl by a microbial community Study Leader: **John R. Lawrence**	Contribution to ***Clean Environment; Toxics Result*** *of EC's management framework*

Diclofop-methyl is a post-emergence herbicide used mainly in the prairies to control the growth of annual grasses in cereal crops. Persistence of this chemical has been observed in groundwater and rivers. An experimental microbial community to degrade diclofop-methyl was established as a biofilm. In this rehabilitation, changes in population structure were measured using denaturing gradient gel electrophoresis (DGGE) during growth of the biofilm under different nutritional conditions. Nine individual bacterial isolates obtained from the biofilm were studied under the same conditions in order to identify the isolates in the community samples. Population banding patterns obtained by DGGE changed as the biofilm aged. Nutritional conditions during biofilm development were also found to influence the banding patterns. A number of the observed bands, that appeared to correspond to individual isolates, were extracted from the gel and nucleotide sequence analysis was performed. Other bands in biofilm samples could not be linked to bands from the nine isolates suggesting that there might be non-culturable strains playing a role in biofilm formation or in diclofop degrading activity. These studies demonstrated the utility of molecular approaches for detailed monitoring of population dynamics in degradative microbial communities, and for assessing the response to various environmental factors.

Characterization of Human Impact

Genetically modified organisms in aquatic systems Study Leader: **John R. Lawrence**	Contribution to ***Nature; Ecohealth Result*** *of EC's management framework*

Human impacts were characterized in terms of effects of genetically modified organisms (GMOs) on aquatic systems. Understanding the fate and effects of genetically modified organisms in aquatic systems is increasingly important for regulation, release and potential success of biotechnological applications of bacteria. Studies were carried out to assess the capacity of a surrogate green fluorescent protein (GFP) labeled *P. fluorescens* to survive and integrate into river biofilm communities. Three levels of complexity were examined experimentally to assess the impact of species richness on survival of the surrogate (i) in pure culture, (ii) in combination with selected river biofilm isolates and (iii) in combination with the complete river flora. During biofilm development, observations and monitoring of GFP expression were carried using CLSM, total populations were assessed using the general nucleic acid stain SYTO63, and exopolymer was visualized using lectin staining. In addition, a species specific rRNA probe was used to monitor the abundance of the inoculant. The expression of GFP label was found to be adequate to detect the presence of the inoculant in all three systems during the initial 2-3 weeks of the experiment. However, comparisons with nutrient supplementation prior to imaging of GFP fluorescence indicated that without a pre-incubation period the numbers of GFP labeled cells were underestimated. The organism was still detectable in the river biofilms using the rRNA probe after 3 months of incubation. The use of stable GFP for longer term monitoring of the integration of introduced microorganisms into complex communities may be a viable technique.

Effects of Naturally Occurring Contamination on Aquatic Ecosystems Study Leader: **John R. Lawrence**	Contribution to ***Nature; Ecohealth Result*** *of EC's management framework*

The estimation of effects of naturally occurring contamination on aquatic ecosystems is an important first step in understanding conditions created during industrial development. To assess the impact of hydrocarbons, this study used analysis of planktonic, sediment and biofilm microbial communities in naturally hydrocarbon affected (downstream) and unaffected (upstream) regions of tributary rivers of the R. Athabasca, Northern Alberta. Results showed significant differences in community structure and hydrocarbon degrading activity. Carbon utilization spectra indicated that differences could be detected in only two of three tributaries. Mineralization of ^{14}C naphthalene and hexadecane indicated low levels of activity in planktonic samples, with substantially higher levels associated with both sediment and biofilm communities. These studies also

indicated that an induction period was required before mineralization of naphthalene occurred, in contrast no induction was required for mineralization of hexadecane either upstream or downstream of hydrocarbon deposits. Further, molecular analyses using gene arrays indicated that there were differences at upstream and downstream locations in the presence and absence of genes encoding for enzymes involved in the degradation of a variety of contaminants. These data show a response to the presence of natural hydrocarbon sources at the microbial community level, and potential for the application of molecular analyses of microbial communities for the assessment of environmental effects.

Many of these studies have been conducted in collaboration with partners.

C.W. Greer, NRC, BRI, Montreal/TSRI/STAGE/Molecular analyses/gene arrrays/chips.

R.A. Snyder/University of West Florida, USA/Fate of GMO's in the environment.

P. Maloszewski /GSF/Munich, Germany/Canada Germany collaborative agreement/Transport of Bacteria in the environment.

J.V. Headley/TSRI/Organic analyses/Fate of toxics in biofilms.

D.R. Korber. University of Saskatchewan, Molecular biology/biofilm development.

J.J. Germida, University of Saskatchewan; TSRI/Application of FAME techniques. .

M.J. Hendry, University of Saskatchewan;/Deep subsurface microbiology/gas fluxes/bacterial transport.

UFZ, Magdeburg/Canada Germany collaborative agreement including the following partners:

T. R. Neu, interactions of toxics with microbial biofilms and aggregates;

K. Wendt-Pottoff, applications of molecular tools;

W. Manz, application of molecular tools in microbial ecology; and

M. Winkler, analytical capacity/fate of pharmaceuticals in river systems.

Flocs and colloids in the aquatic environment

Flocculated fine-grained sediment constitutes a complex matrix of microbial communities, organic matter (detritus, cellular debris and extracellular polymers) and inorganic materials. Suspended flocs within any aquatic system play a significant ecological role as they can regulate the overall water quality due to their physical, chemical and or biological activity. Perhaps most importantly, toxic chemicals, including most heavy metals, the majority of priority pollutants and many other unlisted but environmentally sensitive chemicals, have environmental pathways that are primarily associated with inorganic and bio-organic colloids, particles or flocs. There is, however, a fundamental lack of knowledge on the controlling factors of flocculation and how the actual structure of a floc influences its physical, chemical and/or biological behaviour.

Fine sediments play a major role in the transportation of hydrophobic contaminants in river systems. Consequently, a knowledge of their transport characteristics is an essential prerequisite for modelling contaminant transport and interactions with biota. Four studies were conducted in such priority ecosystems as Hamilton Harbour; the St. Clair River, Ontario; and the Fraser River, BC.

Flocs and colloids: structure and behaviour Study Leaders: **Ian G. Droppo** and **Gary Leppard**	Contribution to ***Nature; Ecohealth*** ***Result*** *of EC's management framework*

Hamilton Harbour bed sediment floc.

To improve knowledge of floc and contaminant behaviour, this study focused on the complex structural matrix of natural and engineered flocs. By using correlative microscopic techniques to provide a large range of magnifications, scientists have developed a better understanding of the structural components present and their influence on the functional (settling/transport) aspects of flocculated sediment.

In a MITE project with collaborators from AERB-NWRI, a novel quantitative application of STEM-EDS was used to examine heavy metal contaminant/colloid associations in sediments. Contaminated sediments were analysed to yield microscale information on the accumulation of copper by native iron-rich coatings on the surface of *in situ* sediment bacteria. A special emphasis was placed on copper-contaminated lakes near Sudbury. A novel combination of chemical, physical and electron-optical correlative laboratory technology was employed in a TSRI

project. In this interdisciplinary approach, individual iron-coated bacteria were examined with regard to the iron accumulation process, under well-controlled experimental conditions. These experiments allowed researchers to determined how bacteria and their coatings sorb cadmium and lead from water, prior to contaminant transport, bioavailability changes and transformation.

In joint research, undertaken collaboratively with an NSERC grant, the structure, composition and function relationships in microbial flocs were elucidated. This research has been extended to undertake intrafloc and colloid-specific compartmentalisation analyses of contaminated wastewater flocs. Ongoing research addresses relationships between the bound water content of activated sludge and important engineering parameters like sludge retention time, floc structure and floc surface properties. The effect of sludge retention time on floc surface charge and hydrophobicity was ascertained.

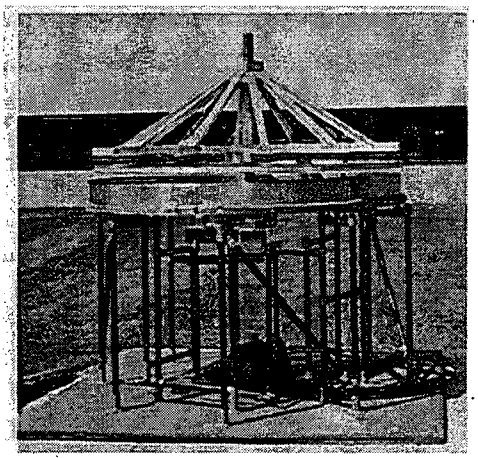
Collaboration with NWRI-Saskatoon, focused on methods development biofilm research. Split samples, made it possible to correlate electron-optical information from the analytical facility in Hamilton with that obtained by the Scanning Confocal Laser Microscopy (SCLM) laboratory in Saskatoon. As a direct result of this approach the resolution limit of the SCLM laboratory has been improved 200-fold. AEPB now has the capacity to localise environmentally interesting molecules, in biofilms, to the EPS level of individual fibrils. Industrial biofilm results continue to reveal a greater integration of biofilm components with industrial membrane structure. This ultrastructural research has been institutionalised within a Canadian membrane filter industry, an external partner.

Many of these studies have been conducted in collaboration with partners. For example, the NSERC Strategic Research Grant to Dr. Ian Droppo included Prof. Steven Liss, Ryerson Polytechnic U., and Prof. Grant Allen, U. of Toronto. An NSERC Major Equipment Grant purchased a confocal microscope for characterizing flocs with Prof. Roger Jacobs et , McMaster U. A MITE grant helped develop electron-optical techniques to analyze the microstructure and chemistry of contaminated sediments. Research on activities of floc parts relevant to metal decontamination was undertaken with Prof. Danielle Fortin, U. of Ottawa, and Prof. Grant Ferris, U. of Toronto under a TSRI Grant. Research on genomics technology for characterizing bacterial communities in flocs was with Dr. Steven Liss, Ryerson Polytechnic U. under a STAGE Grant. The following partnership(s) also contributed to the studies:

1. on structure/function relationships in biofilms with Dr. John Lawrence, Saskatoon.;
2. on floc/contaminant associations in Hamilton Harbour with Dr. Chris Marvin, AERB-NWRI; Prof. Brian McCarry, McMaster U; and Dr. Gary Stern, DFO-Winnipeg;
3. on electron-optics applications to environmental samples with Prof. Larry Arsenault, McMaster-Medicine, and Prof. John Lott, McMaster-Science;
4. on mechanisms of contaminant transformations in sediments with Prof. J.-F. Gaillard,Northwestern U., Chicago, under NSF-USA;

5. on technology development for "per colloid" analyses of colloids which constitute "floc parts".with Profs. Jacques Buffle and Kevin Wilkinson, CABE Environmental Institute Geneva, Switzerland;
6. on electron-optical analysis of contaminant transport in ground waters.with Dr. Didier Perret, U. of Lausanne, Switzerland and Dr. Denis Mavrocordatos, U. of Geneva, Switzerland ; and
7. on adaptation of electron-optics technology to the monitoring of oceanic waters for colloidal indicators of pollution with Dr. Enzo Funari, National Institute of Health, Rome, Italy.

Transport and Erosional Characteristics of Contaminated Bed Sediment in Hamilton Harbour Study Leader: **Ian G. Droppo**	Contribution to ***Nature; Ecohealth Result*** *of EC's management framework*



Annular flume

The Hamilton Harbour, Ontario, Area of Concern (AOC) is characterized by bed sediments which are contaminated primarily by anthropogenic inputs of heavy metals and PAHs. Concerns have been raised that ship traffic in the Harbour could resuspend this contaminated sediment by propeller wash causing eventual contamination of cleaner areas of the Harbour. Experiments were conducted, in the annular flume, to determine the erosion characteristics of contaminated bed sediments from Hamilton Harbour. Controlled flume experiments were also conducted with kaolinite clay. These results showed that the bed deposited had a strong influence on the bed stability. The amount of stress required to erode beds deposited under shear was up to eight times larger than for beds deposited under quiescent conditions. In addition, it was discovered that the presence of biofilm had very significant impacts on the erosion resistance of the bed as well as on the behaviour of the sediment flocs brought into suspension. The results of this study are relevant to any aquatic environment where sediment remobilization is of concern and should be considered in modelling the transport of sediments and associated contaminants.

These studies were conducted with various partners and collaborators including :
Ryerson Polytechnic University (S. Liss) and University of Toronto (G. Allen), who collaborated on studies of floc structure and behaviour. K. Irvine, State University of New York (SUNY) in Buffalo, NY who collaborated on studies of street sediments, CSOs and stormwater runoff.

In-situ Size Distribution of Suspended Sediment Particles in the Fraser River System Study Leader: **B.G. Krishnappan**	Contribution to ***Nature; Ecohealth Result*** *of EC's management framework*

A submersible laser instrument, was used to measure the size distribution of suspended sediment flocs at a number of cross-sections throughout the length of the Fraser River. These results demonstrated that suspended sediment particles in the river are transported as flocs rather than as individual particles, and the flocculation of the sediment particles has occurred due to the effluents from the pulp mills and other sources. Concurrent measurements of the flow field have shown that the size of the sediment flocs is governed by the flow turbulence level. For example, at a transect near Lilloet, BC, where the river has the highest slope and fastest currents, the flocs were broken up into primary particles, whereas at a transect near Mission, BC, where the slope of the river is mild and the currents are low, the flocs are the largest with a median diameter of 75 μm (by comparison, the primary particle's median size is only 10 μm).

Fine Sediments in the St. Clair River: Transport Characteristics and the Risk of Resuspension by Ship Traffic Study Leader: **B.G. Krishnappan**	Contribution to ***Nature; Ecohealth Result*** *of EC's management framework*

The St. Clair River sediments were studied in the Rotating Circular Flume to measure the critical shear stresses for erosion and deposition, and transport characteristics. These results will be used for modelling the transport and fate of contaminants in the river sediments. The results of a battery of tests showed that the resuspension characteristics of bed sediments depend on the shear stress history during deposition and the time of consolidation. In these studies, part of the deposition occurred under a shear stress below the critical stress that would prevent deposition while the rest of the deposition occurred under quiescent conditions. The latter condition resulted in a fluff layer that was weakly dependent on the floc size at the end of applied shear history. Flocs deposited under an applied shear had a greater resistance to resuspension than the fluff layer. The practical importance of this finding is that sediment resuspension is highly dependent on depositional history, particularly, if the conditions lead to a large fluff layer.

The International Joint Commission has identified the St. Clair River as an Area of Concern, and elevated levels of mercury and chlorinated hydrocarbons have been found in the river sediments from Sarnia, Ontario to Lake St. Clair. The contaminated sediments are found in 4 to 6 m of water adjacent to the shipping channel on the Canadian side of the River. The deposition zones in the St. Clair River normally act as sinks for the contaminants; however, during the passage of large ships [Class 7 and 10], the Bernoulli wave increases the local shear stress by more than a factor of 2, causing it to exceed the critical shear

stress for resuspension, thus converting the 'deposit' into a potential source of contaminants. Other causes of resuspension include increased river flow due to high wind setup on Lake Huron, and direct propeller wash. Mass movement of contaminated sediment may also occur due to ice scouring. The purpose of this research is to determine the critical shear stress and resuspension potential of the sediments in one of the depositional zones of the St. Clair River, and whether the temporal response of the sediment would be rapid enough to be affected by the ship passage.

These experiments showed that sustained application of high shear stress could cause a change in the bed form and abrupt change in the resuspension.

Much of this research was carried out jointly, in partnerships with the University of Windsor and the University of New Orleans, USA.

Characterization of Urban Wet-Weather Pollution

In wet weather, additional sources of urban pollution are activated and impact on the receiving water ecosystems through discharges of stormwater and combined sewer overflows (CSOs). Several types and sources of such pollution were primarily studied in two Areas of Concern, Toronto Waterfront and Hamilton Harbour.

Urban runoff conveys heat from impervious surfaces like roofs, roads, and parking lots by contributing to thermal pollution of receiving waters. This pollution impacts on aquatic habitat with potential succession for cold water species to warm water species and concurrent loss of aquatic biodiversity.

Thermal pollution of Urban runoff Study Leader: **Jiri Marsalek**	Contribution to ***Nature; Ecohealth*** ***Result*** *of EC's management framework*

To predict urban runoff temperatures, a mathematical model was developed, using a thermal energy balance and a one-dimensional heat equation for surface temperature of, and temperature gradient in, asphalt during wet and dry weather periods. Runoff temperature was estimated as a function of rainwater temperature and surface temperature of the asphalt. Computer simulations of pavement temperatures compared well with actual measurements of temperature in a test plot. Actual rainfall events were supplemented with simulated events, using rain making equipment, to enhance the volume of experimental data. The mathematical model developed can be used to predict thermal loading conveyed by runoff from impervious areas. In wet weather, additional sources of urban pollution are activated and impact on the receiving water ecosystems through discharges of stormwater and combined sewer overflows (CSOs). Several types and sources of such pollution were studied primarily in two Areas of Concern, Toronto Waterfront and Hamilton Harbour.

Combined Sewer Overflows and Stormwater Runoff in Hamilton Harbour

In order to develop effective remedial programs, the inputs and fate of contaminants to Hamilton Harbour need to be assessed. This study focused specifically on the Kenilworth sewershed, a residential, commercial and heavy industry area, which impacts Hamilton Harbour. The major sources of contaminants include: combined sewer overflows (CSO), coal pile runoff and direct industrial cooling water discharges (monitored by the MISA program of the MOEE). Multiple storm events

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Aquatic Ecosystem Protection Branch

Annual Report

1999/2000

Edited by

R.J. Maguire and J.K. Cooley

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NWRI Contribution No. 00-302

AEPB ANNUAL REPORT 1999/2000

Director's Foreword

It is my pleasure to present this report of research highlights from the Aquatic Ecosystem Protection Branch (AEPB) of Environment Canada's National Water Research Institute for fiscal year 1999-2000. I hope that this report will provide useful information to our numerous governmental, university, private sector, non-governmental organization and international partners and collaborators. The National Water Research Institute is located at the Canada Centre for Inland Waters in Burlington, Ontario and at the National Hydrology Research Centre in Saskatoon, Saskatchewan. Most of the fifty AEPB staff are in Burlington, but four staff members are located in Saskatoon.

Research highlights this year are in the areas of toxic substances fate and effects (including endocrine disrupting substances), early mortality syndrome in Great Lakes salmonids, effects of urban and agricultural land use practices, and biodiversity. These will be discussed in the Project reports below. In addition, the Director of the AEPB is "issue lead" for the Institute in the following areas: (1) toxic chemicals (CEPA PSL, pesticides, Toxic Substances Management Policy Tracks 1 and 2 chemicals, EDSs, Canadian Environmental Quality Guidelines); (2) sustainable agriculture; (3) ecological monitoring; (4) impacts of waterborne toxic substances in the urban environment; (5) St. Lawrence River; (6) Lakewide Management Plan for Lake Ontario; (7) Stream 2 of the Canada-Ontario Agreement; (8) biotechnology; and (9) hazards of microorganisms.

AEPB hosted the Environment Canada Workshop on Potential Ecosystem Effects of Genetically-Modified Organisms on February 28-29, 2000. The purpose of this workshop was to develop a common understanding of Environment Canada's role with respect to research and monitoring on potential ecosystem effects of GMOs, and to identify the next steps required for the Department to fulfill that role. The workshop results will aid in defining the ecosystem science component of an Environment Canada Strategic Plan for Biotechnology.

I thank Mary Jo Scott, Colleen Kennedy, Carol Perry, Suzanne Lesage, Jenn Dykeman and Kristin Alward for their provision of support to the AEPB research programs.

R. James Maguire, Ph.D., F.C.I.C.
Director, Aquatic Ecosystem Protection Branch

Mot du directeur

Il me fait plaisir de présenter ce rapport sur les faits saillants de la recherche au sein de la Direction de la protection des écosystèmes aquatiques (DPEA) de l'Institut national de recherche sur les eaux d'Environnement Canada, pour l'année financière 1999-2000. J'espère qu'il s'avérera utile à nos nombreux partenaires et collaborateurs des organismes gouvernementaux, des universités, du secteur privé, des organisations non gouvernementales et de la communauté internationale. L'Institut national de recherche sur les eaux est logé au Centre canadien des eaux intérieures à Burlington, en Ontario, et au Centre national de recherche en hydrologie à Saskatoon, en Saskatchewan. La plupart des cinquante employés de la DPEA travaillent à Burlington, seulement quatre se trouvant à Saskatoon.

Cette année, les faits saillants de la recherche sont dans les domaines du devenir et des effets des substances toxiques (notamment des substances perturbatrices des systèmes endocriniens), du syndrome de mort précoce chez les salmonidés des Grands Lacs, des effets des pratiques d'utilisation des terres urbaines et agricoles et de la biodiversité. Ces faits saillants sont présentés plus bas dans les rapports sur les projets. De plus, le directeur de la DPEA assume pour l'Institut la responsabilité principale des domaines suivants : 1) produits chimiques toxiques (LSIP de la LCPE, pesticides, produits chimiques considérés dans les Voies 1 et 2 de la Politique de gestion des substances toxiques, SPSE, Recommandations canadiennes pour la qualité de l'environnement); 2) agriculture durable; 3) surveillance écologique; 4) impacts des substances toxiques aquatiques sur le milieu urbain; 5) fleuve Saint-Laurent; 6) plan d'aménagement panlacustre du lac Ontario; 7) Voie 2 de l'Accord Canada-Ontario; 8) biotechnologie; et 9) dangers des microorganismes.

La DPEA a accueilli l'atelier d'Environnement Canada sur les effets potentiels des organismes génétiquement modifiés sur les écosystèmes les 28 et 29 février 2000. Cet atelier avait pour objectif de bien faire comprendre à tous le rôle d'Environnement Canada en matière de recherche et de surveillance concernant les effets potentiels des OGM sur les écosystèmes, et de déterminer les activités que le Ministère devra entreprendre pour remplir ce rôle. Les résultats de l'atelier aideront à définir les activités scientifiques relatives aux écosystèmes du futur plan stratégique d'Environnement Canada concernant la biotechnologie.

Je tiens à remercier Mary Jo Scott, Colleen Kennedy, Carol Perry, Suzanne Lesage, Jenn Dykeman et Kristin Alward pour le soutien qu'elles offrent aux programmes de recherche de la DPEA.

R. James Maguire, Ph.D., M.I.C.C.
Directeur, Direction de la protection des écosystèmes aquatiques

Table of Contents

THE NATIONAL WATER RESEARCH INSTITUTE (NWRI)

"Generating, applying and communicating knowledge for a better environment"

The National Water Research Institute (NWRI) is Canada's largest freshwater establishment. It conducts a comprehensive program of research and development in the aquatic sciences, in partnership with the Canadian and international science communities.

THE INSTITUTE MISSION

Through ecosystem-based research, the National Water Research Institute creates and disseminates new knowledge and understanding of aquatic ecosystems required for the resolution of environmental issues of regional, national or international significance to Canada.

AQUATIC ECOSYSTEM PROTECTION BRANCH (AEPB)

THE BRANCH MISSION

AEPB conducts research to protect aquatic ecosystems from the deleterious effects of toxic chemicals by developing knowledge and understanding of priority pollutants to support informed environmental decision-making and sustainable practices. Studies focus on knowledge requirements of the Toxic Substances Management Policy, the Canadian Environmental Protection Act and the Pest Control Products Act. AEPB research is directed towards a fundamental understanding of such issues as priority chemicals, regional ecosystems, pollution prevention, ecosystem sustainability and biodiversity.

Director	R.J. Maguire
Research Technologist	S.P. Batchelor
Executive Assistant	M.J. Scott
Science Liaison Officer	S. Lesage
Word Processor	J. Dykeman (K. Alward)
Administrative Officer	C. Kennedy
Administrative Clerk	D. Weekes (C. Perry)

Non-Point Sources of Pollution Project
Priority Substances Exposure Project
Priority Substances Effects Project

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L'Institut national de recherche sur les eaux (INRE) est le plus grand établissement de recherche sur les eaux douces du Canada. On y exécute un programme de recherche et développement complet dans le domaine des sciences aquatiques de concert avec les communautés scientifiques du Canada et de l'étranger.

LA MISSION DE L'INRE

Grâce à la recherche écosystémique, l'INRE acquiert et diffuse des connaissances sur les écosystèmes aquatiques qui permettent de résoudre les problèmes environnementaux de portée régionale, nationale ou internationale importants pour le Canada.

DIRECTION DE LA PROTECTION DES ÉCOSYSTÈMES AQUATIQUES (DPEA)

LA MISSION DE LA DIRECTION

La Direction de la protection des écosystèmes aquatiques (DPEA) effectue des recherches dans le but de protéger les écosystèmes aquatiques des effets nocifs des substances chimiques toxiques; pour ce faire, elle cherche à mieux connaître les polluants d'intérêt prioritaire pour la prise de décisions éclairées en matière d'environnement et l'instauration de pratiques respectueuses de l'environnement. Les études sont axées sur les besoins en connaissances liés à la Politique de gestion des substances toxiques, à la *Loi sur les produits antiparasitaires* et à la *Loi canadienne sur la protection de l'environnement.* Les scientifiques de la DPEA contribuent à l'accroissement des connaissances sur des questions comme les substances chimiques d'intérêt prioritaire, les écosystèmes régionaux, la prévention de la pollution, la pérennité des écosystèmes et la biodiversité.

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Projet sur les sources diffuses de pollution
Projet sur l'exposition aux substances d'intérêt prioritaire
Projet sur les effets des substances d'intérêt prioritaire

AEPB Links to Environment Canada's Management Framework.

As an integral part of the NWRI research program, AEPB delivers results that address Environment Canada's Management Framework. The close ties between AEPB research results and EC's Business Lines will be identified throughout the report.

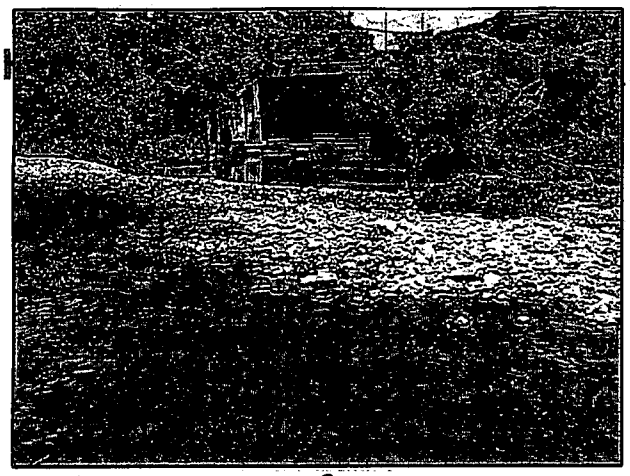
Business line	***Clean Environment***	***Nature***
OUTCOME	*Protection from domestic and global sources of pollution.*	*Conserve biological diversity in healthy ecosystems.*
RESULTS	1.2.0 ***Toxics Result*** *Prevention or reduction of the environmental and human health threats posed by toxic substances and* other substances of concern.	*2.1* ***Eco-Health Result:*** *Human impacts on the health of ecosystems are understood and reduced* 2.2 ***Priority Ecosystems Result:*** *Priority ecosystems are conserved and restored*
SUB-RESULT	*1.2.1Adverse impacts on human health and the environment from existing toxics and other substances of concern are understood by Canadians and prevented or reduced (Existing substances)*	2.1.2 Advance the understanding of the impacts of human activities on the health of ecosystems

Table 1 Environment Canada's Management Framework.

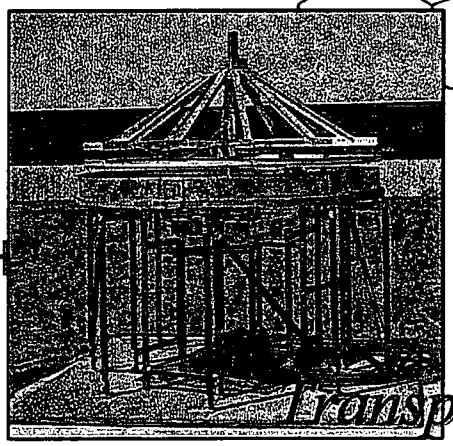
Business Line	**Related Studies**
Clean Environment Toxics Result	• Rotating annular reactors – J.R. Lawrence • Effects of microorganisms on the fate of toxics in river biofilms – J.R. Lawrence • Degradation of Diclofop-methyl by a microbial community – J.R. Lawrence • Toxicity of stormwater and CSOs – J. Marsalek • Reducing pollutant export from agricultural areas – H. Ng • Agricultural best management practices – H. Ng • Environmental toxicology of priority substances and effluents – S. Brown • LC-GC-MS identification of toxic substances – D. Bennie • Development and application of quantitative structure-activity relationships and predictive methods for toxicity data – K.L.E. Kaiser • Fate and effect of contaminants on aquatic ecosystems – T. Mayer

	• Occurrence and fate of priority organic chemicals in environmental samples and effluents – H.-B. Lee • Biodegradation of priority and target chemicals – D. Liu • Xenoestrogenic screening procedure to identify substances of concern – B.K. Burnison • Fate and effects of pesticides and industrial chemicals in water – R.J. Maguire • Transformation of contaminants in wetlands, natural waters and biota – J.V. Headley • Analysis of polar priority organic compounds in aquatic environment – F.I. Onuska
Nature Eco-Health Result	• Genetically modified organisms in aquatic systems – J.R. Lawrence • Effects of naturally occurring contamination on aquatic ecosystems – J.R. Lawrence • Flocs and colloids: structure and behaviour – I.G. Droppo & G. Leppard • Transport and erosional characteristics of contaminated bed sediment in Hamilton Harbour – I.G. Droppo • In-situ size distribution of suspended sediment particles in the Fraser River system – B.G. Krishnappan • Fine sediments in the St. Clair River: Transport characteristics and the risk of resuspension by ship traffic – B.G. Krishappan • Thermal pollution by urban runoff – J. Marsalek • Kenilworth combined sewer outfall (twin 1.75 m square pipes – J. Marsalek • Toxicity of stormwater and CSOs – J. Marsalek • Hydraulics laboratory and research support services – W.B. Taylor • Fate and effect of contaminants on aquatic ecosystems – T. Mayer • Impacts of toxic chemicals and other stresses on the biodiversity of freshwater mussels in the lower Great Lakes drainage basin – J.L. Smith
Nature Priority Ecosystems Result	• Analysis of water quality trends in the Great Lakes system – A.H. El-Shaarawi • Reducing pollutant export from agricultural areas – H. Ng • Reducing pollutant export from urban catchments – B.G. Krishnappan & J. Marsalek • Impacts of toxic chemicals and other stresses on the biodiversity of freshwater mussels in the lower Great Lakes drainage basin – J.L. Smith

Table 2 Summary of AEPB Studies as related to EC's Business Lines

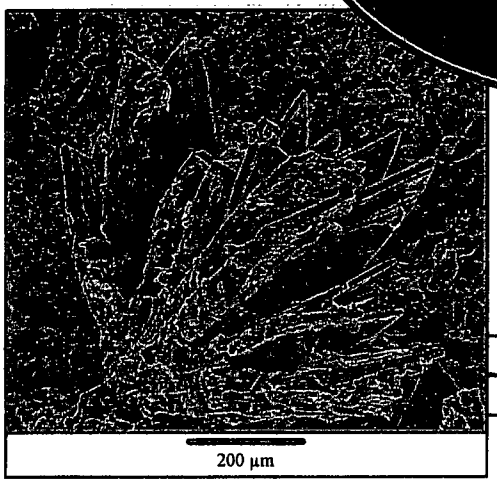


Toxicity of stormwater & CSOs



Transport & Erosion of contaminants

Non-Point Sources of Pollution Project



Non-point source pollution

Flocs & Colloids

were sampled for the CSO and coal pile runoff as well as a wide distribution of street sediments. All samples were analyzed for metals and PAHs. Both the contaminant levels and loads from these sources were evaluated in assessing the potential impact they may have on the success of sediment and aquatic remediation in Hamilton Harbour.

Kenilworth Combined Sewer outfall (twin 1.75 m square pipes) Study Leader: **Jiri Marsalek**	Contribution to ***Nature; Ecohealth Result*** *of EC's management framework*

The inputs to, and fate of contaminants in, Hamilton Harbour need to be assessed prior to development of effective remedial programs. This study focused specifically on the Kenilworth sewershed (a residential, commercial and heavy industry area) impacting Hamilton Harbour. The major sources of contaminants include: combined sewer overflows (CSO), coal pile runoff and direct industrial cooling water discharges (monitored by the MISA program of the MOEE). Multiple storm events were sampled for the CSO and coal pile runoff as well as a wide distribution of street sediments. All samples were analyzed for a host of metals and PAHs. Both the contaminant levels and loads from these sources were evaluated in assessing the potential impact they may have on the success of sediment and aquatic remediation in Hamilton Harbour.

Toxicity of Stormwater and CSOs Study Leader: **Jiri Marsalek**	Contribution to ***Nature; Ecohealth*** *and* ***Priority Ecosystem Results*** as well as **Clean Environment; Toxics result** *of EC's management framework*

Acute toxicity, chronic toxicity and genotoxicity of stormwater and CSOs were studied at 19 urban sampling sites in Ontario, Canada, using a battery of seven bioassays. The observed toxic responses were evaluated by Toxicity Point Values, corresponding to various levels of toxicity and ranging from non-toxic to severely toxic. Most frequent responses of severe acute toxicity were found in stormwater samples (in 14% of all samples), particularly those collected on freeways during the winter months. Compared to stormwater, combined sewer overflows (CSOs) displayed lower acute toxicity (7% of samples were moderately toxic), and none of the samples was severely toxic. The frequency of genotoxicity detection in CSOs was higher than acute toxicity detection (15% of samples were at least moderately genotoxic). Up to two thirds of all CSO samples showed chronic toxic effects, depending on the toxicity test applied. In receiving waters, however, such observed effects may be offset by mixing and dilution with less polluted ambient water over the corresponding seven-day period of the chronic test. Toxicity measurements were found effective in screening and comparing sources of toxicants, but their effectiveness in prediction of control performance and the assessment of chronic toxicity has not yet been demonstrated. Toxicity of UV disinfected treated wastewater effluents was reported in a journal paper. No significant indications of UV-induced toxicity were found in the effluent samples

tested, regardless of the location, or intensity of UV disinfection. Thus, UV did not appear to increase effluent toxicity, which may occur with chlorination/ dechlorination and potentially harm the receiving water ecosystem.

The assessment of impacts of urban stormwater and CSO discharges on receiving waters using benthic communities offers advantages for describing spatially extensive impacts (covering large sections of receiving waters) with response time scales in the order one month. Consequently, this methodology should be useful in RAP areas in the Canadian Great Lakes Basin, with respect to assessing the existing conditions, planning restoration, and assessing restoration results. Towards this end, collection of benthic data from two AOCs (Toronto and Hamilton) has been continued. Ten field sites were assessed earlier and described in a paper under preparation, and new sites were added in this fiscal year. New sites included an outfall from the North Toronto CSO treatment facility, the Kingston Pond (a well documented site), the Dunkers CSO storage facility in Scarborough, Oshawa Creek (an impacted urban stream currently investigated by other EC agencies), and Farewell Creek (a smaller stream east of Oshawa, offering possibilities for studying both local and cumulative impacts). Preliminary results indicate that while contaminant levels (heavy metals and PAHs) were relatively high in exposed sediments, biological effects appeared to be minimal. Toxicity of sediments was low, and alterations in benthic communities small. Neither toxicity endpoints nor benthic community descriptors were related to sediment contaminant levels. Future research will focus on a different experimental design (upstream/ downstream sampling), and study sites with minimal variability of habitat conditions.

This work was undertaken in partnership with Great Lakes 2000 Cleanup Fund (S. Kok); Queen's University Stormwater Quality Enhancement Group (W.E. Watt, B.C. Anderson and M. Van Buren), joint research on stormwater impacts on receiving waters (including thermal pollution; another partner - The Regional Municipality of Ottawa-Carleton, M. Trudeau) and stormwater treatment; BETO (P. Seto), The City of Toronto (P. Chessie) joint research on CSO treatment and impacts of CSOs on receiving waters; Environmental technology companies/associations: Centennial Concrete Pipe and Products, Ltd., Munro Concrete Products, Ltd. and the Ontario Concrete Pipe Association, cost recovery research and development of new products; NATO and Hydroinform, Inc., Prague, Czech Republic, collaboration on staging advanced research workshops on environmental issues of current interest (a workshop on floods completed, another one on pollution source controls is under preparation).

Analysis of Water Quality Trends in the Great Lakes System Study Leader: **A.H. El-Shaarawi**	Contribution to ***Nature; Priority Ecosystems Result*** *of EC's management framework*

Water quality trends were investigated with respect to the Niagara River and Lake Ontario. The modelling of spatial and temporal trends in the Niagara River contaminant concentrations and loads was undertaken for a 10-year period from 1986/87 to 1996/97. The results show that considerable progress has been achieved in reducing concentrations of 99 different chemicals found in the River. Studies of Lake Ontario focused on three tasks: 1) assess the long-term water quality response of the lake to the P control program, 2) evaluate seasonal trends in key water quality parameters and 3) evaluate more recent whole-lake spatial data to determine whether *Dreissena* mussels have affected water quality. The water quality parameters addressed include the macro-nutrients mentioned above as well as such parameters as Chl, diatom biomass and water transparency. The sources of data analyzed for seasonal and long-term trends were two monitoring programs initiated on Lake Ontario to follow the response of the lake to reductions in P loading. The Surveillance Program (Environment Canada, Burlington Ontario) examined such key variables as TP, soluble reactive P, nitrate, silica and Chl, during lakewide cruises which begun in the early 1970s. The program results track these key variables from the era before phosphorus load reductions, which were initiated in the mid-1970s. The results will appear as a chapter in a book in the Eco-S.

These studies were conducted with collaboration from Ontario and Atlantic Regions and staff from McMaster University.

Reducing pollutant export form agricultural areas Study Leader: **Howard Ng**	Contribution to **Nature; Priority Ecosystems Result and Clean Environment; Toxics *Result*** *of EC's management framework*

Controlled drainage (CD), and controlled drainage combined with subsurface irrigation (CDS), were assessed with respect to nitrate concentration, nitrate loss, tile drainage volume, water table depth, soil moisture content and crop yields, by comparing them with free drainage (FD). Three field sites, two with clay loam soils and one with sandy loam soils, were established in Southwestern Ontario. Each field site was divided into two plots. The CD, CDS and FD systems, incorporated with conventional tillage (CT) and no-tillage (NT), were operated in experimental paired plots. On the clay loam sites, the CD with NT reduced the mean nitrate concentration and loss by 10% and 26%, respectively, whereas the CD with CT reduced the mean nitrate concentration and loss by 24% and 14%, respectively, compared to FD. At the sandy loam site, the CDS with CT reduced the flow-weighted mean nitrate concentration and nitrate loss by 38% and 37%, respectively, compared to FD. The CD with NT or CT reduced the total tile drainage volume by 18% and 23%, respectively,

compared to FD. The tile drainage volumes for CDS and FD, at the Bicrel site, were almost equal.

The CD with NT or CT increased the average total soil moisture content by 6% and 7%, respectively, whereas the CDS increased the total soil moisture content by 23%, compared with FD. The increase of soil moisture content resulted from additional subsurface irrigation of the CDS plot. The CD with NT or CT raised the mean water table depth by 1% and 3%, respectively, compared with FD, and the CDS treatment effectively raised the mean water table depth by 39% (average of 1995 and 1996), compared to FD. The CD with NT has not affected the soybean yields, and the with CT increased the soybean yields just by 3%, compared with the corresponding FD plot. The CDS increased tomato yields by 11% in 1995 and corn yields by 64% in 1996, compared to the FD treatment.

These investigations were undertaken in collaborative partnership with C.F. Drury, C.Tan and J.D. Gaynor of AAFC, Harrow, Ontario (On-farm evaluation of controlled drainage and subsurface irrigation technologies) and Professor R. Rudra, University of Guelph, Ontario(evaluation of nitrogen level).

Agricultural Best Management Practices Study Leader: **Howard Ng**	Contribution to ***Clean Environment; Toxics Result*** *of EC's management framework*

At the Chevalier and Shanahan sites, compost was applied to the controlled drainage plots in the fall of 1998. Both Chevalier and Shanahan farms were planted with soybeans during the 1999 cropping season. Tile drainage samples collected from September 1998 through April 1999 from the controlled drainage plots with compost were analyzed for nutrients, major ions and trace metals. The analytical results showed that concentrations of nutrients (N, P, K) and major ions (Ca, Mg, Cl, Na), respectively, were 2-3 times and 1.5-2 times higher, and trace metals (Pb, Zn, Cu, Cd, Fe) were 2-4 times lower, compared with the results from the same plot without compost application. Evaluation of the effect of compost on tile drainage water quality continues.

At the Bicrel site, sweet peas were planted in early April, 1999 and harvested in early June, 1999. Soybeans were seeded after the harvest of sweet peas. Due to extended warm weather, the Bicrel farm produced two crops during the cropping season. The extension of cropping frequency led to an increased amount of fertilizer and pesticide input to the soil. The tile drainage and rainwater samples collected prior to the field season were processed and analyzed. An assessment of dry weather impacts on farm water quality continues.

Analytical results for NO_3 -N, TP and K in rainwater and tile drainage samples were analyzed and summarized for the years of 1997 through 1999. The mean (1977-1999) concentrations (mg/L) of NO_3 -N, TP and K for the rainwater and the tile drainage, respectively, were 1.175, 1.160 and 0.077, and 0.254, 0.041 and 0.046. All three studied nutrients showed higher

concentrations in the rainwater than in tile drainage. A study report is being prepared.

Reducing contaminant export from urban catchments Study Leaders: **B.G. Krishnappan** and **J. Marsalek**	Contribution to ***Nature; Priority Ecosystem Result*** *of EC's management framework*

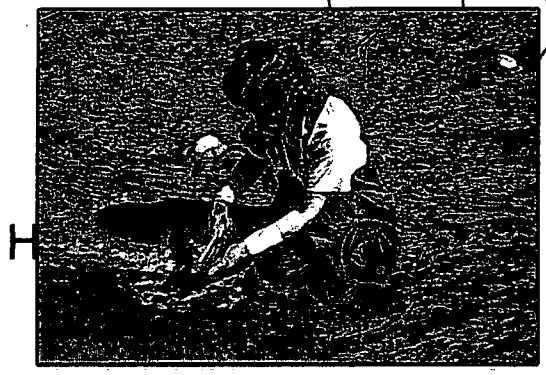
To advance the understanding of suspended solids removal in stormwater management ponds, transport characteristics of the sediment deposited in an on-stream stormwater management pond in Kingston, Ontario, Canada were studied in the rotating circular flume. In deposition experiments, a known mass of sediment, mixed with a known volume of pond water, was placed in the flume and subjected to a quasi-constant bed-shear stress generated by operating the flume at various speeds of rotation. At each speed, the concentrations of sediment in suspension and the size distributions were monitored as a function of time. From these data, the critical shear stress for deposition (τ_{cd} = 0.050 N/m^2) and the amount of sediment that would stay in suspension permanently were established. In erosion tests, the pond sediment was first allowed to deposit on the flume bottom over a period of 41 and 138 hours at a shear stress slightly below the critical shear stress for deposition. The erosion characteristics of these deposits were studied by applying bed-shear stress in step increments. At each step, the concentration of eroded sediment and its size distribution were measured as a function of time, and the observed data were used to estimate the critical shear stress for erosion of the surface sediment layer (estimated as 0.121 N/m^2). The erosion tests also provided quantitative data on the amount of sediment that would be re-suspended for a given bed-shear stress. Finally, empirical relationships were developed for deposition and erosion and recommended for modelling fine sediment transport in the pond studied.

This work was undertaken in collaboration with Prof. Alex McCorquodale, University of New Orleans, USA who collaborated on the St.Clair River sediment study; and Profs. Graf and Altinakar of Swiss Federal Institute of Technology at Lausanne, Switzerland who collaboration on the development of a 2D flow and sediment/contaminant transport model.

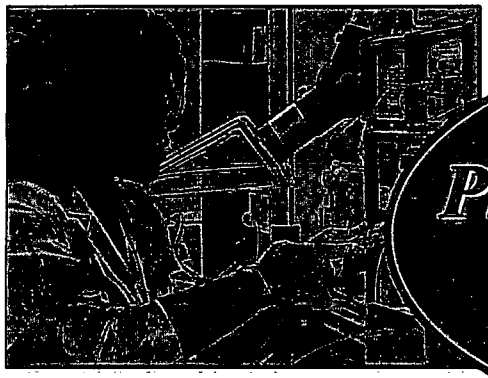
Hydraulics Laboratory and Research Support Services Study Leader: **W.B. Taylor**	Contribution to ***Nature; Ecohealth Result*** *of EC's management framework*

Effective delivery of quality ecosystem science, based on management of S&T capacity and infrastructure consistent with federal S&T policy by providing specialized hydraulic, sediment transport, and air/water interaction laboratory resources and expert advice to support NWRI research programs. Technical resources were directed to NPSP Project Studies, and advice and support was provided to research staff in other NWRI Branches, Department of Fisheries and Oceans and the University of Guelph.

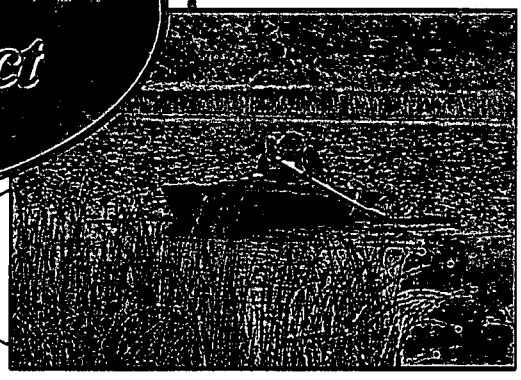
Reproduction & Development

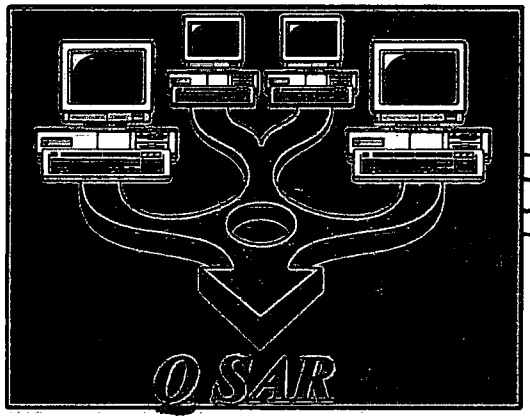


Biodiversity

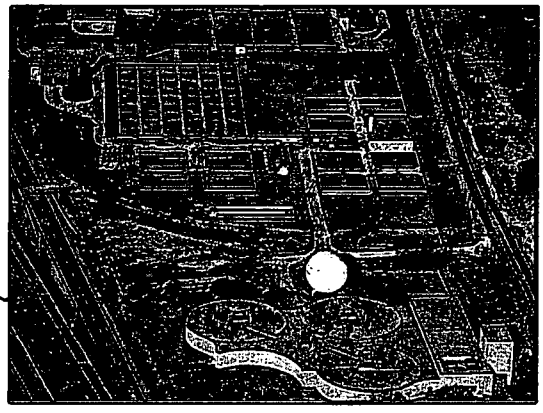


Priority Substances Effects Project





Effects and Fate of CEPA PSL & Other Priority Substances



Priority Substances Effects Project, Fiscal year 1999-2000
Project Chief: Scott Brown

The Project provides important scientific information on the effects of priority substances on aquatic organisms in support of Departmental hazard and risk assessments, and risk management activities. This knowledge is used by Environment Canada to make decisions under various initiatives including: the Toxic Substances Management Policy, the *Canadian Environmental Protection Act*, the *Pest Control Products Act* and Priority Ecosystems Initiatives (*e.g.*, Great Lakes 2000, Georgia Basin Ecosystem Initiative). The Project accomplishes its goals by developing and applying techniques to assess priority substances and effluents for their potential to cause impacts on the survival, growth, development and reproduction of aquatic biota, and by investigating fundamental mechanisms governing the persistence and fate of toxic chemicals in aquatic ecosystems.

Current activities include determining the effects of chemicals on the reproduction and development of fishes, the biodiversity of aquatic ecosystems, the development of structure-activity relationships using neural networks and related artificial intelligence methods in support of rapid chemical assessment, and the environmental impacts, persistence and fate of high priority chemicals such as nonylphenol, endocrine-disrupting substances, road salts and polynuclear aromatic hydrocarbons.

Project Chief	S.B. Brown
Study Leaders	D.T. Bennie
	B.G. Brownlee
	K.L.E. Kaiser
	T. Mayer
	J.L. Smith

Research Technologists	M. Brown
	G.A. MacInnis
	V. Palabrica
	I. Scott
	C.A. Sullivan
	M. Villella

Effets des substances d'intérêt prioritaire, année financière 1999-2000
Responsable : Scott Brown

Ce projet permet d'obtenir d'importants renseignements scientifiques sur les effets des substances d'intérêt prioritaire sur les organismes aquatiques, renseignements que le Ministère utilise dans ses évaluations des risques et des dangers et dans ses activités de gestion du risque. Environnement Canada applique ces connaissances dans ses prises de décisions effectuées dans le cadre de diverses initiatives, notamment : la Politique de gestion des substances toxiques, la *Loi canadienne sur la protection de l'environnement*, la *Loi sur les produits antiparasitaires*, et les initiatives concernant les écosystèmes prioritaires (p. ex. Grands Lacs 2000, l'Initiative de l'écosystème du bassin de Géorgie). Pour atteindre les objectifs de ce projet, on élabore et applique des techniques permettant de déterminer les effets potentiels des substances d'intérêt prioritaire et des effluents sur la survie, la croissance, le développement et la reproduction des organismes aquatiques, et on étudie les mécanismes fondamentaux régissant la persistance et le devenir des substances chimiques toxiques dans les écosystèmes aquatiques.

Au nombre des activités en cours, on compte la détermination des effets des produits chimiques sur la reproduction et le développement des poissons, l'étude de la biodiversité des écosystèmes aquatiques, l'établissement de relations entre structure et activité au moyen de réseaux neuronaux et de méthodes d'intelligence artificielle connexes permettant une évaluation chimique rapide, et l'étude des impacts environnementaux, de la persistance et du devenir des substances chimiques d'intérêt hautement prioritaire, comme le nonylphénol, les substances perturbatrices des systèmes endocriniens, les sels de voirie et les hydrocarbures aromatiques polycycliques.

Responsable	S.B. Brown
Chefs d'études	D.T. Bennie
	B.G. Brownlee
	K.L.E. Kaiser
	T. Mayer
	J.L. Smith
Technologues de recherche	M. Brown
	G.A. MacInnis
	V. Palabrica
	I. Scott
	C.A. Sullivan
	M. Villella

Environmental Toxicology of Priority Substances and Effluents: Early and Sensitive Indicators of Toxic Syndromes

This Study investigates the toxicological mechanisms of deleterious substances on fish growth, reproduction and development. It also develops new techniques to identify and monitor endocrine modulating capability, responses and effects of priority substances and effluents in aquatic biota in support of national priorities under the Clean Environment Business Line (e.g., TSMP, CEPA [PSL 1 & 2] and Great Lakes initiatives [COA Targets 2.1 for tier 1 and tier 2 substances]). Additionally, the Study produces new knowledge in support of national priorities under the Nature Business Line (e.g., the rehabilitation of degraded populations of native species [COA Target 1.3.3] and protecting the function and structure of diverse, self-sustaining biological communities [COA Target 3.31]). The aims of the project are: a) to understand the progression of toxicity of high-priority pollutants from the molecular level to effects at the whole fish level, b) to identify biochemical, endocrine and physiological responses in exposures to contaminants which will prove useful in studies on wild fishes. Changes in reproductive, developmental, hormonal or biochemical function often precede, but could also result from disturbed physiological processes. It is thus conceivable that toxic chemicals will disrupt these systems early in the development of toxic syndromes. Therefore, it is anticipated that early and sensitive indicators of toxicity may be obtained, along with insight of toxic mechanisms, by studying the possible interference of toxic pollutants in these systems. After responses are validated, techniques and knowledge are applied to natural fish populations.

Environmental Toxicology of Priority Substances and Effluents Study Leader: **Scott Brown**	Contribution to ***Clean Environment; Toxics Result*** *of EC's management framework*

Investigations of the adverse effects of priority effluents and their bio-active components on processes essential for reproductive competence, growth and development in fishes.

Results suggest that exposure to environmentally relevant concentrations of both nonylphenol and estrogen may compromise successful parr-smolt transformation, which is critical to long-term growth and survival of salmon at sea. The results will contribute to understanding factors which may affect sustainability and management of Canadian and international stocks of Atlantic and Pacific salmon. The work will also be used for risk management of alkylphenols under the current CEPA PSL II assessment. NWRI scientists Brown, Bennie, Burnison & Sherry; in partnership with Fairchild and Haya,DFO and University partners Eales, UM, and MacLatchy, UNB replicated effects of aquatic estrogenic substances to determine critical time windows for exposure to fish.

The project evaluated possible mechanisms whereby certain priority substances and effluents disrupt the known actions of thyroid hormones and

antioxidant vitamins. Knowledge of these processes could lead to the development of new and specific indicators of toxicity. For example, imbalances in the metabolism of retinoids can produce levels of metabolites which are potentially genotoxic and teratogenic. The presence of these metabolites may serve as useful indicators of adverse effects. The metabolites as well as the lipophillic contaminants may be parentally transferred to offspring and create deleterious effects on early life stages. In 1999/2000 work revolved around assessing thyroid function in Atlantic salmon smolts exposed to estrogen and nonylphenol. Our previous studies have shown that fish exposed to pulp and paper mill effluent and polychlorinated organics in both the lab and field have depressed gonadal growth and steroid secretion concomitant with reductions in hepatic retinol stores. In collaboration with D. Alsop (Ph.D. student) and G. Van der Kraak, University of Guelph, testied the hypothesis that local generation of retinoic acid plays a fundamental role in ovarian development in goldfish and rainbow trout. Progress has been made in association on a HPLC method to separate and quantify all-trans retinoic acid, 9 cis-retinoic acid and retinol. In the longer term, understanding the factors influencing ovarian retinoic acid metabolism, the mechanism of action of retinoic acid and how this relates to the expression of its nuclear receptors.

Early-life stage mortality in salmonids and other species is a concern because it represents a serious impediment to the restoration and maintenance of sustainable populations in the lower Great Lakes. Investigations on temporal and spatial variabilty in the micronutrient dynamics of prey species from the lower Great Lakes and investigations to assess the efficacy EMS treatment protocols were completed. This project now in its second year included the following collaborators: Brown, EC and Fitzsimons, DFO; Honeyfield and Tillitt USGS-BRD; Michigan and Wisconsin DNRs; and Wright, COFTMA. Progress reports have been submitted regarding these experiments and sample analysis, and S. Brown organized and chaired a basin-wide meeting on Early Mortality Syndrome. He also co-chaired a special session at the International Council for the Exploration of the Sea, Annual Science Conference.

Identification of toxic substances in environmental samples.

Research continued in the environmental occurrence, fate and effects of nonylphenol and nonylphenol polyethoxylates as well as other endocrine disrupting substances in natural waters, textile mill effluent and municipal sewage treatment plant effluent.

Alkylphenol polyethoxylate surfactants are widely used in a variety of commercial, industrial, institutional and household formulations in Canada. These surfactants are used in textiles manufacturing, pesticide formulations, petroleum refining, leather processing, plastics manufacture, spermicidal preparations and numerous other applications. These varied uses offer many routes into the environment for these surfactants. Since most municipalities in Canada have some type of sewage treatment facilities, waste effluents generated from processes that make use of these surfactants are generally treated before discharge to the environment. These sewage treatment plants (STPs) play a significant role in the transformation and degradation of the surfactants into more toxic and weakly estrogenic metabolites. Under aerobic and anaerobic STP conditions, the parent alkylphenol polyethoxylates are transformed into short chain alkylphenol ethoxylates, alkylphenoxy carboxylic acids and alkylphenols. Release of these substances to the environment can occur by effluent discharge and by disposal of sludge generated in the treatment processes. This generated sludge may be disposed of by incineration, landfilling or spreading onto agricultural soil.

LC-GC-MS Identification of Toxic Substances Study Leader: **D.T. Bennie**	Contribution to ***Clean Environment; Toxics Result*** *of EC's management framework*

Environmental occurrence and fate of nonylphenol and its polyethoxylates.

Studies to determine the environmental occurrence and fate of nonylphenol and its polyethoxylates continued with the collection and analysis of samples of various effluent streams of municipal sewage treatment plants, textile mills and pulp and paper mills in Atlantic Canada. The alkylphenolic parameters determined were 4-nonylphenol (4-NP), nonylphenol ethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO), nonylphenoxyacetic acid (NP1EC), nonylphenoxyethoxyacetic acid (NP2EC), nonylphenol polyethoxylates (NPnEO, where n = 3 to 17), 4-*tert*-octylphenol (4-*t*-OP), octylphenoxyacetic acid (OP1EC) and octylphenoxyethoxyacetic acid (OP2EC). These studies addresses research needs identified for the CEPA PSL 2 assessment of nonylphenol and its ethoxylates as well as departmental issues regarding endocrine disrupting compounds.

Seawater adaptability, growth and survival of Atlantic salmon smolts.

In collaboration with other researchers at Environment Canada and the Department of Fisheries and Oceans, a study was undertaken to determine the effects of water-borne 4-nonylphenol on the seawater adaptability, growth and

survival of Atlantic salmon (*Salmo salar*) smolts. A recent study found significant relationships between historical applications of an insecticide containing 4-nonylphenol (4-NP) and catch data for Atlantic salmon 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. Field work was conducted on the Miramichi River in northeast New Brunswick to ascertain the levels of alkylphenolic substances discharged from sewage treatment plants and pulp mills to which Atlantic salmon smolts might be exposed during the parr-smolt transformation phase. The chemical analysis work on these samples is not complete at this time. This study addresses research needs identified for the CEPA PSL 2 assessment of nonylphenol and its ethoxylates as well as departmental issues regarding endocrine disrupting compounds.

Chlorinated paraffins (SMCCPs) in marine mammals and freshwater fishes

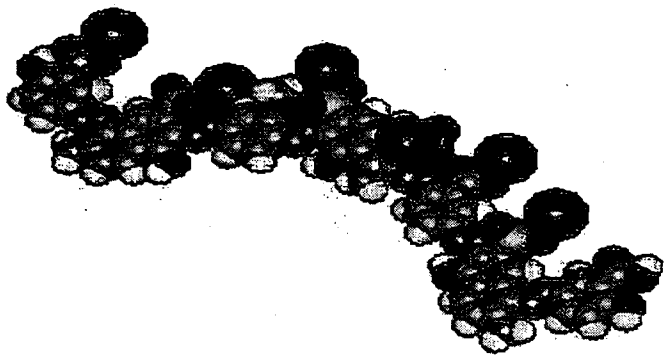
This study measured the levels of short and medium chain chlorinated paraffins (SMCCPs) in marine mammals and freshwater fishes, chlorinated paraffins (CPs) were on the first Priority Substances List (PSL 1) of CEPA. It was not possible to assess whether these substances were "toxic" as defined under Paragraph 11(a) of CEPA because no data were identified on their concentrations in the Canadian environment. Short-chain CPs are currently under consideration for classification as Track 1 Priority Toxic Substances under the federal Toxic Substances Management Policy. This study addressed the need for data on the levels in the aquatic environment, particularly in biota. During the study, samples of twenty-five dead beluga whales (*Delphinapterus leucas)* from the St. Lawrence River estuary, as well as samples of ten rainbow trout (*Oncorhynchus mykiss*) and three carp (*Cyprinus carpio*) caught in western Lake Ontario were analyzed for total short (C_{10}-C_{13}) and medium (C_{14}-C_{17}) chain chlorinated paraffins using gas chromatography low resolution negative chemical ionization mass spectrometry. Short and medium chain chlorinated paraffins were quantitatively identified using two commercial preparations. SMCCPs were

detected in all samples. Results ranged from 1.1 to 59 μg/g wet weight in beluga liver tissue, from 6.4 to 166 μg/g wet weight in beluga blubber and from 0.41 to 9.7 μg/g wet weight in the two freshwater species. The beluga results are higher than those reported for marine mammals in Europe. Total short and medium chain chlorinated paraffin levels in the beluga blubber are comparable to previous ΣPCB and ΣDDT results for the same population of beluga whales. The mean SMCCP concentration in the carp was 0.90 μg/g wet weight and 2.7 μg/g wet weight in the rainbow trout. Results from the freshwater species are comparable to those reported for fish taken from other industrially impacted waterways in North America but are substantially elevated relative to marine species from European studies.

Development and application of quantitative structure-activity relationships and predictive methods for toxicity data Study Leader: **Klaus L.E. Kaiser**	Contribution to ***Clean Environment; Toxics Result*** *of EC's management framework*

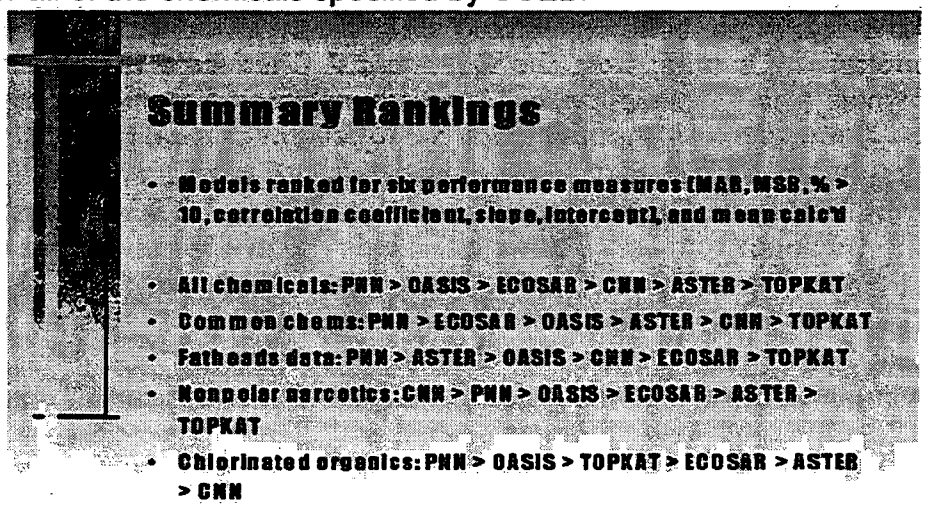
Research progressed on the development and application of neural network methodology for the prediction of acute toxicity values for fish and other aquatic species. We have now adapted the probabilistic neural network (PNN) system was adapted to two other endpoints, namely Daphnia magna and Tetrahymena species' LC50 values. Several reports arising from this work have been submitted for publication. Network input was modified to account for larger molecules and a group of over 1,000 substances were started to be evaluated. Due to the complexity of many of these substances (larger size molecules, as found in many dyes) their structure files contained numerous errors and which needed to be corrected first, consuming a significant amount of time. This work was supported by a grant from the Commercial Chemicals Evaluation Branch (CCEB), which also specified the 1,000 substances for which the toxicity is being predicted. A description of the methodology is given in several publications and a detailed report to CCEB, also available as NWRI Technical Note. A detailed report by an independent contractor, commissioned by CCEB, compares the predictions obtained from our PNN methodology with the results from several other methodologies and found our PNN to be overall superior.

There are over 20,000 substances on the Domestic Substances List and over 45,000 substances on the Non-Domestic Substances List, most of which are individual substances with clearly identifiable chemical structures and toxicological and physical properties. Most of the existing software (ASTER, OASIS-1,OASIS-2, ECOSAR, TOPKAT, CNN) cannot provide any prediction at all for many of these substances, due to their large molecular size and complexity (Fig. 1). Furthermore, where these models do provide answers, they are often wrong by several orders of magnitude.



The figure above shows the Chemical structure of a typical "Domestic Substance (Non-Confidential)", CAS RN 84963-14-4, C58H41N15O24S4.xNa; benzoic acid, 2-[[1-amino-7-[[4-[[6-amino-5-[(3-carboxy-4-hydroxyphenyl)azo]-1-hydroxy-3-sulfo-2-naphthalenyl]azo]-2-methoxyphenyl]azo]-8-hydroxy-3,6-disulfo-2-naphthalenyl]azo]-5-[[6-amino-5-[(3-carboxy-4-hydroxyphenyl)azo]-1-hydroxy-3-sulfo-2-naphthalenyl]azo]-, sodium salt. The PNN computed acute toxicity to fathead minnow is 11 mg/L.

In order to compare these methodologies with the Probabilistic Neural Network (PNN) methodology developed here, a separate contract let by CCEB has been evaluating the results from the different methodologies for a set of chemicals as supplied by CCEB. Results of this work were presented at the SETAC Conference, Nov. 1999. The summary rankings are given below. It should also be noted that the PNN was the only method to provide quantitative results for all of the chemicals specified by CCEB.



Summary rankings for various computational methods on the basis of prediction results for a list of test substances provided by CCEB.

This work was undertaken in collaborative partnership with: CCEB on the prediction of acute toxicities of chemicals; P.C. Jurs, Pennsylvania State University; K.M. Gough, University of Manitoba; R. Kuhne/G. Schuurmann, UFZ, Germany on Prject 059; and T.W. Schultz, University of Tennessee.

Fate and Effect of Contaminants on the Aquatic Ecosystems Study Leader: **T. Mayer**	Contribution to ***Nature; Ecohealth result and Clean Environment; Toxics result*** *of EC's management framework*

Wetland Research

A comprehensive review of wetland research in Canada was conducted to identify research gaps and to highlight the importance of preserving these ecosystems. Research on both natural and constructed wetland systems was reviewed. Natural wetlands are an important part of Canadian landscape providing habitat to flora and fauna and contributing significantly to the Canadian economy. The review revealed a need for better knowledge on the fate and transport of urban and agricultural pollutants to better understand the effects of land use on the water quality and health of wetlands. Effective management of these ecosystems can not be assured without improved knowledge of wetland hydrology and hydrogeochemistry. A better understanding of the effect of climate change on wetlands will lead to better preservation. The literature survey reported a more limited use of wetland treatment technology in Canada than in the U.S. This indicated that it is timely to generate knowledge of cold weather performance, design adaptation, and the effect of wetlands on wildlife, before these technologies gain widespread acceptance in Canada.

Research on nutrient sources at Point Pelee identifyied internal nutrient cycling as the principal source of nutrients to the Sanctuary Pond, one of the six major open-water ponds at Point Pelee National Park. Studies showed thatnearshore Blue Heron area of Point Pelee Marsh was likely impacted by sewage-derived input, possibly from contaminated groundwater.

New research on the biogeochemistry of prairie wetlands was initiated. The research is concerned with the quality of sediment porewater in the prairie potholes or sloughs which are a source of solutes to groundwater. In the semi-arid northern prairies, groundwater plays an important role in sustaining the vegetation in surrounding wetlands, which are a major breeding area of North America's waterfowl.

Research on wetlands was carried out in partnership with other NWRI study leaders, C. Ptacek, A. Crowe, and R. Bourbonnniere who investigated the role of groundwater in nutrient transport in wetlands. Parks Canada contributed financially and logistically to the project several years ago. Research on the effect of urban contaminants on the aquatic ecosystems was conducted in collaboration with J. Marsalek, M. Servos, and support staff from NWRI.

Effect of road salts on aquatic ecosystems

Road salts have been identified as a class of contaminants, which may have adverse effects on the environment. As a result, road salts were included on the second Priority Substances List (PSL2) under CEPA. As a part of the environmental risk assessment, information essential for the exposure characterization in surface waters was assembled. This information consisted of data on chloride concentrations in Canadian surface waters and information on the fate and effects of road salt constituents on freshwater ecosystems. Results of an investigation of a small urban detention pond located in the Rouge River Valley were incorporated in the report. This pond receives high loadings of road salts and is a reasonable surrogate for small urban lakes and ponds. The data revealed that small water bodies, with limited dilution capacity in large urban watersheds, receiving high salt loadings are most susceptible to impairment. Identification of surface waters most sensitive to the impact of road salts has important implication for road salt management strategies.

Research on the fate, transport and effects of the priority substance, Road salts, involved collaboration withU. Borgman, NWRI, B. Snodgrass, formely with MTO, and D. Morin of CCEB, EC. and financial support provided by the CCEB, EC.

Impacts of toxic chemicals and other stresses on the biodiversity of freshwater mussels in the lower Great Lakes drainage basin. Study Leader: **J.L. Smith**	Contribution to ***Nature; Eco-health and priority ecosystem results*** *of EC's management framework*

Studies on the conservation status of rare and endangered mussels in southwestern Ontario continued, with a focus on determining strategies for restoring mussel populations in the Sydenham River and Lake St. Clair. A study to determine changes over time in the diversity and distribution of freshwater mussels in the Grand River was completed, and a similar study on the Sydenham River is nearing completion. Status reports on three mussel species at risk were completed, and reports on two additional species are in preparation. The preliminary phase of a study to investigate the genetic relationships of mussels across the Lake Erie/Ohio River drainage divide was concluded, and plans are underway for a larger study in FY 2000-01. A multi-agency project to develop a recovery plan for the Sydenham River, which is an important refuge for many rare and endangered aquatic species, was initiated.

Toward the development of strategies for re-habilitating/re-establishing freshwater mussel populations in southwestern Ontario.

The decline of freshwater mussels in southern Ontario rivers may be due to a variety of factors, including poor water quality, the loss fish hosts, and habitat alteration or destruction, whereas the loss of mussels from Lake St. Clair is largely because of the impact of the Zebra Mussel, *Dreissena polymorpha*. The long-term goal of this research is to develop strategies for protecting and recovering the mussel communities of southern Ontario. The objectives of this year's project were to evaluate water quality and fish communities as factors limiting the distributions of mussels in several rivers, determine the demographics of the healthiest remaining mussel communities in the Sydenham River to use as recovery targets for the system, determine microhabitat preferences of various species, and identify the biological traits that determine a species' survivability in stressed environments and its potential for recovery.

Water quality data collected in 1998 from 66 mussel survey sites on the Grand, Thames, Sydenham, Ausable and Maitland Rivers showed that there were significant differences in water quality within and among the rivers. There were also distinct differences in both water quality and the composition of the mussel community between the east and north branches of the Sydenham River, suggesting several possibilities for cause/effect links. Historical water quality data from the Ontario Ministry of the Environment for the Sydenham River (1965-1996) were examined for trends over time, but were of limited used due to numerous information gaps. A list of recognized fish hosts for 36 native mussel species was extracted from the literature, and the most likely hosts for Ontario populations of nine candidate species at risk were determined by comparing distribution patterns (and changes over time in these patterns) of mussels and fishes. A model used to predict mussel diversity from drainage area and fish diversity in the Ohio River drainage was applied to southern Ontario rivers. Results showed that the Ausable and Sydenham rivers support more species of mussels than expected, indicating that they are unusually rich "mussel" rivers. A quantitative survey for mussels at two productive sites in the Sydenham River showed that densities were similar (3.3 and 3.0 mussels/m^2) but species composition differed significantly. Density of the endangered Northern Riffleshell at one site (0.25/m^2) was comparable to densities reported for the healthiest remaining populations in North America. There were associations between some habitat variables (depth, substrate) and the diversity and abundance of mussels at these sites. An examination of biological traits showed that mussels that are more obese in shape, sexually dioecious, and have more glochidial hosts increased in abundance or remained relatively stable after zebra mussels invaded Lake St. Clair. Rare species tended to be smaller, required more specific substrates, and had fewer known fish hosts. Shallow areas in the St. Clair River delta serve as refugia from the zebra mussel. Transplant experiments revealed the importance of ensuring that relocated species be placed in appropriate substrates. Future work will focus on defining the chemical and physical attributes of refuge areas in Lake St. Clair, conducting laboratory

tests to confirm the host fishes for rare species, and determining the habitat requirements for these species.

Changes over time in the diversity and distribution of freshwater mussels in the Grand River, southwestern Ontario.

The Grand River, a major tributary to Lake Erie in southwestern Ontario, historically supported a diverse and abundant freshwater mussel fauna, with 33 species recorded from the system since 1885. A recent examination of species occurrence records from the Grand River suggested that the mussel community has declined over time, such that fewer species are now present and those that remain tend to be the most pollution-tolerant. This study provided an in-depth assessment of changes over time in the diversity and distribution of mussels in the Grand River, by comparing the results of surveys conducted at a total of 94 sites in 1995 and 1997-98, with those from a survey conducted 25 years earlier and with the historical data. Timed search surveys were conducted in both 1995 and 1997-98, using sampling efforts of 1.5 and 4.5 person-hours, respectively. Only 17 of 33 historical species had been found alive in 1970-72, probably because of the impacts of sewage from a rapidly-growing human population. At that time, only six species survived in the lower reaches of the main stem. Mussel populations have since rebounded, with 25 species found alive throughout the system in 1995 and/or 1997-98, including 21 in the previously impoverished lower reaches. This recovery is attributed to significant improvements in water quality over the past 25 years. The recent addition of fishways to some of the dams and weirs on the river should improve the future reproductive success of mussels, by eliminating barriers to the free movement of their host fishes. Although environmental conditions in the Grand River appear more favourable now for mussels than they have in decades, the population in the watershed is projected to grow by 300,000 over the next 25 years and there are serious concerns that the river may not have the capacity to assimilate the additional wastewaters. If so, water quality, mussel diversity, and the health of the aquatic ecosystem in general, will decline again.

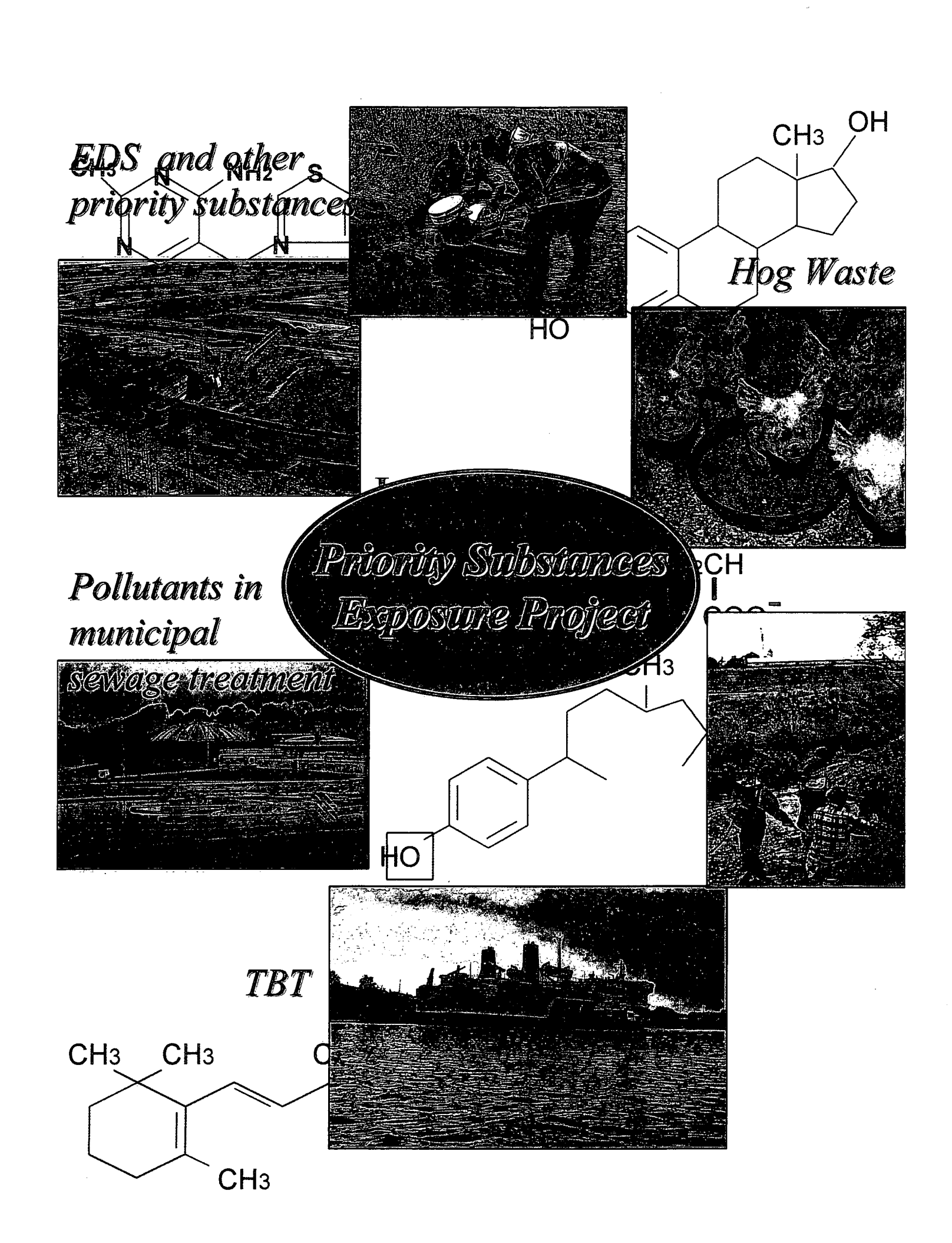
Genetic variation across drainage divides: implications for the restoration of extirpated populations of freshwater mussels.

Populations of freshwater mussels in Lake Erie, its drainage basin, and some parts of Ohio have been decimated by poor water quality, the loss of fish hosts, habitat alteration, and zebra mussels. As aquatic habitats are rehabilitated, the reintroduction of mussels has been proposed to restore extirpated populations. Large populations capable of serving as sources for reintroduction are found in Canadian tributaries to the Great Lakes and parts of the Ohio River. It is not known if these two regions contain genetically distinct populations, and no research has been conducted to date on the genetic or evolutionary implications of the large-scale transport of mussels across drainage basins. Objectives of this project were to: quantify genetic structure of populations of the Threeridge, *Amblema plicata*, and the Mucket, *Actinonaias ligamentina*, from Great Lakes and Ohio River populations (the latter is of

particular interest because it has been extirpated from Ohio); compare patterns of variation between species, and among populations and basins for each species; and provide a preliminary assessment of the feasibility of using these populations as sources for reintroduction of mussels to decimated areas.

Amblema plicata were collected from the Sydenham and Ausable rivers in Ontario, St. Joseph River in Ohio and Michigan (Great Lakes tributaries), and the Ohio, Muskingum, Licking and Tennessee Rivers (Ohio basin tributaries), while *A. ligamentina* were collected from the Sydenham and Thames rivers in Ontario and the Licking River in Kentucky. Samples of mantle tissue from at least 20 individuals/population were analyzed for genetic structure using allozyme electrophoresis. Populations of *A. plicata* showed limited gene flow and significant differentiation across drainage divides, with St. Joseph River (Erie tributary) populations more similar to Ohio basin than to Canadian populations. Conversely, *A. ligamentina* showed less overall variation among populations and no differentiation by basin. Implications of these findings are that Canadian populations of *A. ligamentina* may be suitable source populations for reintroducing this species to Ohio streams, but Canadian and Ohio River populations of *A. plicata* are genetically distinct and should not be transported across the drainage divide. These contrasting patterns indicate a need for further study of additional species to determine whether either pattern predominates among species in this region. Management agencies must consider patterns of genetic variation when choosing potential source populations for restoration efforts.

Some aspects of the research were undertaken through Partnership with: Gerald L. Mackie, Professor, Department of Zoology, University of Guelph, ON. Dr. David J. Berg, Assistant Professor, Department of Zoology, Miami University, Oxford, OH. on genetic analysis of freshwater mussel populations across the Lake Erie/Ohio River drainage divide.


EDS and other priority substances
Hog Waste
Priority Substances Exposure Project
Pollutants in municipal sewage treatment
TBT

Priority Substances Exposure Project: Fiscal year 1999-2000
Project Chief: Mark Servos

The Project provides important scientific information on the effects of priority substances on aquatic organisms in support of Departmental hazard and risk assessments, and risk management activities. This knowledge is used by Environment Canada to make decisions under the Toxic Substances Management Policy, the Canadian Environmental Protection Act, the Pest Control Products Act and Priority Ecosystems Initiatives (*e.g.*, Great Lakes 2000, Georgia Basin Ecosystem Initiative). The Project accomplishes its goals by researching fundamental mechanisms governing the exposure of organisms to toxic chemicals. Dose-response relationships and environmental impacts are established by identifying active components in complex mixtures, and determining the persistence, fate and bioavailability of toxic chemicals in aquatic ecosystems.

Current activities focus on determining the exposure to aquatic organisms and the impacts of priority substances and potential endocrine-disrupting substances (EDSs) such as alkylphenols, tributyltin, natural and synthetic estrogens, municipal effluents, agricultural and urban runoff. Analytical methods are developed and applied to assess the environmental occurrence, persistence and fate of high priority chemicals such as explosives, amines used in natural gas plants and new agricultural chemicals and antifouling pesticides.

Project Chief	M.R. Servos
Study Leaders	B.K. Burnison
	J.V. Headley
	H.-B. Lee
	D. Liu
	F.I. Onuska
	C. Rouleau
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Research Technologists	S. Batchelor
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	T.E. Peart
	K.M. Peru
	K.A. Terry

Exposition aux substances d'intérêt prioritaire: année financière 1999-2000 [illegible]
Responsable : Mark Servos

Ce projet permet d'obtenir d'importants renseignements scientifiques sur les effets des substances d'intérêt prioritaire sur les organismes aquatiques, renseignements que le Ministère utilise dans ses évaluations des risques et des dangers et dans ses activités de gestion du risque. Environnement Canada applique ces connaissances dans ses prises de décisions effectuées dans le cadre de diverses initiatives, notamment : la Politique de gestion des substances toxiques, la *Loi canadienne sur la protection de l'environnement*, la *Loi sur les produits antiparasitaires*, et les initiatives concernant les écosystèmes prioritaires (p. ex. Grands Lacs 2000, l'Initiative de l'écosystème du bassin de Géorgie). Pour atteindre les objectifs de ce projet, on s'efforce d'élucider les mécanismes fondamentaux régissant l'exposition des organismes aux substances chimiques toxiques. On établit des relations dose-réponse ainsi que les impacts environnementaux en identifiant les composantes actives de mélanges complexes, et en déterminant la persistance, le devenir et la biodisponibilité des toxiques chimiques dans les écosystèmes aquatiques.

Les activités en cours sont centrées sur la détermination de l'exposition des organismes aquatiques et des impacts des substances d'intérêt prioritaire et des substances perturbatrices des systèmes endocriniens (SPSE) potentielles, comme les alkylphénols, le tributylétain, les oestrogènes naturels et synthétiques, les effluents municipaux, et les eaux du ruissellement agricole et urbain. Aussi, on élabore et applique des méthodes analytiques permettant d'évaluer la distribution, la persistance et le devenir dans l'environnement de substances d'intérêt hautement prioritaire comme les explosifs, les amines utilisées dans les installations de traitement de gaz naturel et les nouveaux produits chimiques agricoles et pesticides antisalissures.

Responsable	M.R. Servos
Chefs d'études	B.K. Burnison
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	T.E. Peart
	K.M. Peru
	K.A. Terry

Endocrine disrupting chemicals and other priority substances

During the past few years, extensive research programs have been initiated to determine the presence of man-made chemicals capable of producing endocrine disrupting effects. Estrogenic effects (i.e., feminisation) were emphasised in most studies. Some of the chemical classes, which have been shown to elicit these effects, include alkylphenol ethoxylate surfactants (i.e., nonylphenol ethoxylate), octylphenol, bisphenol A, organochlorine pesticides, PCBs, dioxins, PAHs and phthalates in several industrial effluents, municipal sewage, and non-point sources. These estrogen disrupting chemicals (xenoestrogens) mimic the natural estrogen 17ß-estradiol (E_2) and compete for the binding to the estrogen receptor.

Occurrence and Fate of Priority Organic Chemicals in Environmental Samples and effluents Study Leader: **H.-B. Lee**	Contribution to ***Clean Environment; Toxics result*** *of EC's management framework*

Study of BPA in Canadian municipal and industrial wastewater and sludge.

Bisphenol A (BPA), with an annual production approaching one million tonnes in North America during the late 1990s, is widely used in the manufacture of many plastic products. While it is well established that BPA is a toxic and endocrine-disrupting substance (EDS), little is known about its occurrence in the Canadian environment. A large-scale study of BPA contamination in Canadian municipal and industrial wastewater and sludge was completed. A total of about 200 samples were collected, including those from 27 STPs and 15 pulp and paper mills across Canada, as well as 13 industrial facilities in the Toronto area. BPA contamination was detected in all of the 72 sewage samples, with concentrations ranging from 0.080 to 4.98 µg/L (median 0.329 µg/L) for the influent, and from 0.010 to 1.08 µg/L (median 0.136 µg/L) for the effluent. Of the 36 influent/effluent sample pairs studied, BPA in the influent is removed by the sewage treatment process at a median reduction rate of 68%. Levels of BPA accumulation in sewage sludge, for the 50 samples tested, ranged from 0.033 to 36.7 µg/g, on a dry weight basis. A wide range of BPA concentrations, from 0.23 to 149.2 µg/L, was observed for the wastewater collected from selected industrial facilities in the Toronto area. The more contaminated samples came from the sectors of chemicals and chemical products, commercial dry cleaning, as well as packaging and paper products. Based on these data, on-site releases of BPA by industrial facilities seem to be much more widespread than the National Pollutant Release Inventory (NPRI) database has suggested. While relatively high levels of BPA were found in some of the primary treated effluent collected from the deinking mills, BPA concentrations in the secondary treated effluent of all pulp and paper mills were low, with a range from <0.005 to 0.406 µg/L. Except for the samples derived from a few deinking mills, BPA contamination in pulp and paper mill sludge was either low or undetected.

Sensitive methods were developed for the determination of BPA residues in municipal sewage and sludge samples. BPA in wastewater samples was enriched with a C_{18} solid-phase extraction cartridge, eluted with acetone, and converted into its pentafluoropropionyl derivative. For sludge samples, BPA was acetylated and extracted by supercritical carbon dioxide. In both cases, BPA-d_{16} was used as a surrogate to monitor the extraction efficiency. Final analyses of derivatized sample extracts were carried out by gas chromatography/mass spectrometry operating in the electron impact mode. For water samples, the mean recoveries and standard deviations were 89±6%, 94±4%, and 85±7% at fortification levels of 1, 0.1, and 0.025 µg/L, respectively, with a method detection limit of 0.006 µg/L. For solid waste samples, the mean recoveries and standard deviations were 93±5% and 92±6% at fortification levels of 2.5 and 0.25 µg/g, respectively, and the method detection limit was 0.05 µg/g. For the Canadian samples under investigation, concentrations of BPA ranged from 49.9 to 0.031 µg/L in sewage influent and effluent and from 36.7 to 0.104 µg/g in sludge.

Occurrence of EDC in environmental samples.

A new collaborative study of environmental samples was initiate with the City of Toronto. The study determined the occurrence and fate of EDCs such as nonylphenol and its related compounds, BPA. as well as 17ß-estradiol and its metabolites. In addition to influent, effluent, and sludge samples collected from the four sewage treatment plans in Toronto, wastewater samples from selected industries in the Toronto area were also included in this study. The test samples showed a wide range of concentrations varying from over 100,000 µg/L (for nonylphenol ethoxylates in industrial wastewater) to ca. 1 ng/L (for E_2 in some sewage final effluent). This work, which has identified some major industrial sources of EDCs in the Toronto area, will be continued in the next FY.

Biodegradation of 17β-estradiol.

The naturally occurring estrogen, E_2, is one of the most potent EDCs ever studied. However, there is little information in the open literature on the fate of this and other estrogens in the environment, a fact that hinders the assessment of their ultimate impact on the environment. Aerobic and anaerobic batch experiments involving a E_2-degradation culture and a supernatant of activated sludge from a local sewage treatment plant (Burlington, Ontario) were undertaken to assess the persistence of E_2 and its 5 metabolites. The batch experiments showed that E_2 and the metabolites were not persistent and could be rapidly degraded by sewage bacteria. Biodegradation of E_2 by sewage bacteria appeared to initiate at the D ring of E_2, leading to the formation of the major metabolite estrone (E_1). No other major degradation products were noted. However, during the very early stages of E_2 degradation by sewage bacteria, a new metabolite with a lactone structure was also observed. With this new metabolite, a metabolic pathway of E_2 by sewage bacteria was proposed. The study results suggested that the risk of extensive accumulation of natural estrogens normally found in sewage effluents in the environment was small, due to their ready and quick biodegradation.

Greg Gris of Industrial Waste and Stormwater Quality part of Toronto Works. Toronto Works is responsible for sampling and NWRI is responsible for chemical analysis and data interpretation. The data generated will be used to generate new knowledge on endocrine disrupting chemicals, and used by the City of Toronto to establish bylaws for wasterwater quality.

Biodegradation of Priority and Target Chemicals Study Leader: **D. Liu**	Contribution to ***Clean Environment; Toxics result*** *sub result 1.2.1 of EC's management framework*

Fate and persistence of 17β-estradiol (E2) in sewage

There is little information in the literature on the fate of natural estrogens in the environment and this hinders the assessment of their ultimate impact on the ecosystem. A study was conducted to assess the persistence of E_2 and its 5 metabolites using a E_2-degrading bacterial culture and a supernatant of activated sludge from a local sewage treatment plant. The results indicate that the risk of extensive accumulation of natural estrogens normally found in sewage effluents in the environment is small, due to their ready and quick biodegradation. A previously unreported metabolite X1 (5-hydroxy-15-methyl-13-oxatetracyclo[8.7.0.0<2,7>.0.<11,15>]heptadeca-2(7),3,5-trien-14-one) was also identified, thus allowing the elucidation of E_2 degradation mechanism and pathway in sewage. The study results will contribute to understanding of factors which may affect the persistence of natural estrogens in the aquatic environment. The work is relevant for risk management of chemicals with estrogenic activity under CEPA PSL II assessment.

Xenoestrogenic Screening Procedure to Identify Substances of Concern Study Leader: **Kent Burnison**	Contribution to ***Clean Environment; Toxics result*** *sub result 1.2.1 of EC's management framework*

The yeast estrogen system (YES) has been developed in order to quickly measure the estrogenicity of an effluent. The YES assay has been used, in this project, to determine the estrogenicity in selected sewage treatment plant effluents from across Canada. The bioassay was used as the biological endpoint in chemical fractionation procedures to determine specific causative compounds. The YES assay has also proven useful in determining the estrogenicity of the hog farm waste that is sprayed on agricultural lands.

Analytical support was provide to EPS with the CEPA Assessment of Textile Mill Effluents by analyzing samples from a variety of mills from Ontario for estrogenic activity using the YES bioassay.

Municipal sewage effluents

The TIE fractionation on the Toronto Main STP has been repeated because of difficulties encountered with the Milli-Q water systems found in both

NWRI and other labs. Estrogenic contamination was found when one litre of this water was used. If we can maintain the volume of Milli-Q to 100 ml or less, there is not a contaminant problem in the HPLC fractionation. 10 litres of Galt STP effluent has recently been processed by the TIE procedure. The sample was treated through C_{18}-disks and then the non-adsorbed eluent through a C_{18}-column. YES estrogenicity was present in the disk concentrate and the column eluent. An unknown estrogenic peak appeared immediately before the estradiol peak.

Bioassay guided chemical analysis

A study was conducted to investigate the advantages of using short-term bioassays guided chemical analysis for identification of toxic substances in environmental samples. In partnership with university (University of Guelph) and private industry (Grace Bioremediation Technologies), experiments were carried out in laboratories and fields to test the application of bioassay guided chemical analysis in the areas of bioremediation and water quality management. The results show that bioassay guided chemical analysis is highly efficient in the rapid identification of toxic substances in environmental samples. After modification, the test has the potential to be used in the assessment of hot spots of contaminated sites, thus speeding up the remediation action.

Hog Waste

Some farmers in southern Ontario spray their fields with hog waste during late April. The run-off from these fields may be effecting fish reproduction downstream from the field tile drainage. In collaboration with Dr. Andreas Hartmann and Dr. Thomas Ternes (ESWE, Wiesbaden, Germany) studies determined that equol is a major estrogenic compound in hog waste. Equol is a phytoestrogen and a known degradation product of daidzein and formononetin. The estrogenic activity in hog waste was analyzed from an additional three farms including field drainage tiles, and an up and down-stream sample. Upstream sampling from one farm gave a negative result for the yeast estrogen screen (YES) bioassay prior to a rainfall effect but the sample was positive after a rainfall event. Downstream sampling from this farm were negative pre- and post-rain. Tile drainage samples were negative after the rainfall and did not flow prior to the rain. Hog waste from this site was estrogenic, but much lower than the 700-sow site studied in 1998. We are currently trying to locate a source of a yeast culture containing the androgen receptor plus we are sending samples to Dr. Van der Kraak's laboratory at Univ. of Guelph for androgenicity testing.

Dissolved Organic Matter and Cadmium

Two German scientists, Dr. Thomas Meinelt and Mr. Hans-Jürgen Exner, spent three weeks in September, under the of the Canada/Germany Bilateral

Agreement on Ecosystem Health. Investigations continued in partnership on the role of dissolved organic matter (DOM) on the uptake of cadmium into zebrafish embryos. The experiments measured the uptake of ^{109}Cd in the presence of DOM and calcium.

Fate & effects of antifouling pesticides in the environment

Organometals are a concern because of their distribution, persistence and potential toxicity in the aquatic environment. Studies have been focused on understanding the exposure, bioavailability and effects of organometals on aquatic organisms to support the management of these compounds in the Canadian environment. Tributyltin compounds (TBT) have been used as antifouling compounds because of there unique biocidal activity. Studies on the toxicity of TBT compounds to non-target organism, especially in harbours has led to a proposed restriction of their use by the year 2003. Several national surveys for tributyltin have been conduced in order to determine the exposure of these substances to the Canadian environment and to monitor for the effectiveness of remedial actions. Irgarol 1051 is a new replacement chemical for tributyltin being considered for registration under Canada's Pest Control Products Act. Irgarol 1051 is also very toxic to aquatic organisms and there is little information on the ecotoxcity of Irgarol or its degradation product M1, especially in the Canadain environement, a fact that hinders the assessment of its ultimate impact on the environment. Recent studies have shown that M1 has an environmental persistence much greater than that of the parent compound Irgarol 1051, and that M1 still possesses a strong biocidal activity. These results strongly suggest that both Irgarol 1051 and its persistent degradation product M1 may potentially affect and/or damage the primary producer community in aquatic ecosystems. Although Irgarol 1051 and its degradation product M1, has been detected in other countries such as Japan it was not detected in Canadian coastal ports. A better understanding of the exposure and effects of these substances in the environment with result in for effective management and remedial actions for protection of the Canadian environment.

Fate and Effects of Pesticides and Industrial Chemicals in Water Study Leader: **R.J. Maguire**	Contribution to ***Clean Environment; Toxics result*** *sub result 1.2.1 of EC's management framework*

Research continued the effects of tributyltin on freshwater benthic invertebrates such as *Hyalella azteca*. This work is in collaboration D.G. Dixon, University of Waterloo. The third national survey for tributyltin in the Canadian environment was started this fiscal year.

Toxicity of sulfonylurea herbicides to aquatic plants.

Two wetland species, *Mimulus ringens* and *Bidens cernua*, two terrestrial species, *Sinapis arvensis* and *Phaseolus vulgaris*, and one species found in both wet and dry habitats, *Echinochloa crusgalli*, were exposed to 1% (0.045 g-ai/ha) and 10% (0.45 g-ai/ha) of recommended label rate of metsulfuron methyl, a sulfonylurea herbicide widely used in western Canada. The objective of the study was to investigate the effect of metsulfuron methyl on the vegetative growth and reproductive parts of these plant species and to determine the most

sensitive phenological stage. Chemical analyses of the amount of herbicide sprayed were performed using gas chromatography (fiber glass papers) and high performance liquid chromatography (tank mix). There is a good correspondence between the quantity measured in the tank mix compared to that detected on fiber glass papers, the latter representing the dose reaching the plants during the spray event. All species exhibited marked effects on the vegetative growth and reproductive performance when sprayed at 10% label rate. Less pronounced but significant effects were shown at 1% label rate on all species except for *E. crusgalli* where only slight visible injury was noted. Seed weight was reduced for *B. cernua* and *S. arvensis*. Species belonging to the Fabaceae and the Brassicaceae families, represented by *P. vulgaris* and *S. arvensis,* would be notably at risk from small doses of metsulfuron methyl drifting away from the sprayed areas although the two wetland species also exhibited substantial sensitivity to metsulfuron methyl. Several endangered species belonging to these two families are found in or near potholes of the prairies in western Canada. The seedling stage was the most sensitive period for all species tested although surviving plants sprayed at later stages showed considerable effects on the reproductive parts. This study highlights the shortcomings of the current testing schemes required prior to pesticide registration.

The study has been undertaken in partnership with CWS

Biodegradation of Priority and Target Chemicals Study Leader: **D. Liu**	Contribution to **Clean Environment; Toxics result** *sub result 1.2.1 of EC's management framework*

Studies to determine the occurrence and fate of Irgarol 1051 in Canadian and Japanese aquatic environments continued. A related study to assess the ecotoxicity and phytotoxicity of Irgarol 1051 was conducted. A study to investigate the persistence and degradation pathway of 17β-estradiol in sewage was conducted, and a study to examine the feasibility of using short-term bioassay guided chemical analysis for environmental samples was also completed. Highlights of these studies are summarized below:

In a second phase of the joint Canada-Japan research initiative on new antifouling chemical, a two-year survey was conducted in 1998-1999 to investigate the occurrence and fate of Irgarol 1051 in Canadian and Japanese aquatic environments. A total of 6 large trade ports (Vancouver, Toronto, Montreal, Halifax, Mizushima, Kobe) and numerous marinas and fishery harbours were surveyed. Again, Irgarol 1051 was not detected in the Canadian aquatic environment, but Irgarol and its degradation product M1 were found in the enclosed coastal waters of the Seto Inland Sea and the Kii Peninsula just outside of the Seto Inland Sea in Japan. Irgarol 1051 and M1 were not found in the freshwater of Lake Biwa.

This work generates, for the first time, vital information on ambient concentration levels of M1, a stable Irgarol degradation product, in natural aquatic environment. Such information is currently not available in the open literature, the results of this study have been communicated to Environmental Assessment

Division of Pest Management Regulatory Agency for its consideration in the registration of Irgarol 1051 in Canada under the Pest Control Products Act.

Although, Irgarol 1051 is a new replacement chemical for tributyltin there is little information in the open literature on the ecotoxcity and phytotoxicity of Irgarol and its degradation product M1, a fact that hinders the assessment of its ultimate impact on the environment. This study shows that M1 has an environmental persistence much greater than that of the parent compound Irgarol 1051, and that M1 still possesses a strong biocidal activity. These results strongly suggest that both Irgarol 1051 and its persistent degradation product M1 may potentially affect and/or damage the primary producer community in aquatic ecosystems.

This work generates vital information about the ultimate environmental persistence, ecotoxicity and phytotoxicity for Irgarol 1051 and its persistent degradation product M1. Since this information has no been available the hazard assessment for Irgarol 1051 as well as impeding the development of management strategy for its control has been hindered.

Irgarol 1051 has been recently registered in the United States as a replacement biocide for organotins in antifouling paints, and is also being seriously considered for registration under Canada's Pest Control Products Act.

Toxic substances in aquatic environments

Research was completed in support of field programs on the natural attenuation of toxic substances in wetland environments, effects of oilsands derived chemicals on the ecology of northern rivers, and the degradation of pesticides and related chemicals in riverine biofilms and aquatic insects. This entailed the development of analytical technique for measurement of polar amine and organosulphur compounds along with their transformation products; and refinement of laboratory procedures for the determination of the sorption of toxic chemicals to soils and biofilm materials. Major activities are summarized below:

Transformation of Contaminants in Wetlands, Natural Waters and Biota Study Leader: **J.V. Headley**	Contribution to ***Clean Environment; Toxics result*** *of EC's management framework*

A report was completed of an investigation of wetland sites in Alberta. The report was approved by the Associationtion of Petroleum Producers. Results indicate that while the natural wetlands offer a cost-effective means for removal of amines, in general the levels of DIPA and sulfolane (two process chemicals) measured in wetland vegetation at a sour-gas processing facility, appear to fall below reference values recommended for the protection of wildlife. Work was conducted in support of a new initiative "Assessment of Natural and Anthropogenic Impacts of Oil Sands Contaminants Within Northern River Basins". Preliminary field and laboratory studies were completed to characterize oil sands-derived hydrocarbon contaminants in sediments and water in the Athabasca River basin.

Studies on the Fate of gas-condensate in contaminated groundwater has continued in collaboration with the Department of Civil Engineering, University of Saskatchewan. The results to date show that alkanolamines in groundwater can have cosolvency effects on BTEX hydrocarbons and hence alter their solubility and movement in groundwater. There is also evidence that complex mixtures in sour gas-condensate can display non-ideal behaviour in contaminated groundwater. Site specific investigations are therefore needed to assess the fate and transport of such hydrocarbon contaminants in subsurface environments.

Analysis of Polar Priority Organic Compounds in Aquatic Environment Study Leader: **F.I. Onuska**	Contribution to ***Clean Environment; Toxics result*** *of EC's management framework*

The occurrence of 3,3'-dichlorobenzidine in industrial wastewater and a novel extraction procedure

Research was completed in the occurrence and fate of aromatic amines especially 3,3'-dichlorobenzidine in industrial wastewater and municipal sewage treatment plant effluent and sludge samples.

To improve an extraction step from aqueous matrices a novel approach has been studied by applying solid state extraction cartridges. Extraction was carried out using Visiprep manifold from SUPELCO Company. This methodology allows to extract up to 150 mL filtered water sample using PORAPAK-RDX sorbent (500 mg). The cartridge was rinsed with 10 mL of methylene chloride. Residual solvent was removed from the cartridge under vacuum for 10 minutes. Afterwards, the cartridge was conditioned with 15 mL of acetonitrile and 30 mL of organics free water. Immediately, after conditioning was completed, 100 mL of filtered effluent sample was spiked with the surrogate standard containing know amount of 1,5-diaminonaphthalene (3 μg/mL) and was introduced onto the top of the sorbent and sample was extracted through the cartridge at 10 mL/min. After passing the sample through the bed of the sorbent, it was rinsed with 2 mL of water to remove interferences. Elution of analytes from the bed was carried out with 10 mL of acetonitrile. The recoveries for aromatic amines were between 81 to 99 percent.

In co-operation with Dr. D. Liu, we have beeen studying degradation of Dominion Colour Ltd. YELLOW 1242 pigment using sludge from the Humber River Sewage Treatment Plant in Toronto, Ont. Degradation of pigment, which is 3,3'-dichlorobenzidine based molecule indicates that only a very negligible amount of 3,3'-DCB can be accounted for in both anaerobic and aerobic sludge system. We have been following the disappearence kinetics of the pigment during approx. 3 months.

Fifteen pigment samples were obtained from Dominion Colour and analyzed for unreacted benzidines. Samples were analyzed using HRGC-MS as their trifluoroacetyl derivatives and underivatized using isotachophoresis in tandem with capillary zone electrophoresis. The results confirmed presence of free 3,3'-DCB. A short report was provided to Mrs. Edwina Lopez, Environmental Contaminants and Nuclear Program Division-Ontario Region.

Analysis of high energetic materials

Research was completed on the evaluation of accelerated solvent extraction (ASE) for extracting RDX, HMX, TNT and tetryl from sediment samples. This technique combines elevated temperatures, pressure and time significantly reduced for achieving excellent extraction recoveries. The solvents

we used were those normally used for standard extraction of explosives such as acetone, methanol and acetonitrile.

We report that recovery of above explosives from spiked sediment after 5 weeks of equilibration time are quantitative. The extraction time for up to 3 gram sample is less than 20 minutes and the volume of solvent is less than 5 times that of extraction cell containing sample. No evidence was observed for thermal degradation during the extraction of these temperature sensitive samples. An optimized method based on the statistical experimental design was carried out. A paper has been written in co-authorship with Dr. A. El-Shaarawi.

Gas chromatographic method and quantitation of RDX and HMX was studied employing an electron capture and mass spectrometric detectors. Results confirmed that it is possible to obtain very good separation and reliable quantitation of these high energetic materials even they are very thermally sensitive, when a high flow velocities are used, and reduction of active sites in the injector and the inner wall of a capillary is used. Variety of stationary phases such as OV-1701, SE-%2XL and SPB-1 very tested. Tubings having different i.d. from 0.18 to 0.53 mm were tested. A 10 meter long 0.32 mm i.d. SPB-1 column was the most successful one having 0.4 μm film thickness and using split/splitless injection. Hydrogen was used as a carrier gas at 5 mL/min. Responses for all explosives were very good and ranged at low picogram. HRGC-MS in electro ionization mode and negative ion chemical ionization mode was also successful.

Microcystin toxins are produced as secondary metabolites by *Microcystis aeuregiosa*. Mycrocystin LR is a cyclic heptapeptide with MF of 996, mycrocystin RR having MF 1038 and Microcystin YR has MF 1045, which is Thyr-Arg analog of Microcystin LR.

In co-operation with Scott Kirby, we cultivated and extracted microcystins from the biomass provided by him and we developed a suitable extraction technique and quantified microcystin LR, which was the major product in the very complex mixture. Its concentration was 7875 ppm based on dry weight basis. A report is being prepared.

Emerging Issues in Aquatic Ecosystems Protection Branch

Genetically modified organisms

A new study was initiated in collaboration with Agriculture/Agri-Food Canada to evaluate the possibility of gene transfer from transgenic canola crops to wild relative. Transgenic Bt-GFP (insecticidal- fluorescent) canola seedlings were obtained from Dr. Neal Stewart from the University of North Carolina at Greensboro. The project is multidisciplinary and was funded until March 2002. This year was a planning and initiating phase. It is too early to see results.

The study above is entirely a collaboration. Drs. John Lawrence and S. Lesage are involved for NWRI, Drs. Suzanne Warwick, Lorraine Braun, Peter Mason and Anne Légère for Agriculture Canada, and Dr. Neal Stewart for the University of North Carolina. Philip Macdonald represents CFIA.

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APPEXDIX 1

Acronyms found in the text

AEPB	Aquatic Ecosystem Protection Branch
AERB	Aquatic Ecosystem Restoration Branch
AFM	atomic force microscopy
AOC	area of concern
ASE	Accelerated solvent extraction
BPA	Bisphenol A
BTEX	Benzene, toluene, ethyl benzene, and xylene
CCEB	Commercial Chemicals Evaluation Branch
CD	Controlled drainage
CDS	Controlled drainage combined with sub-irrigation
CEPA PSL	*Canadian Environmental Protection Act* Priority Substance List
CFIA	Canadian Food Inspection Agency
CLSM	confocal laser scanning microscope
COC	colloidal organic carbon
CP	chlorinated paraffins
CSO	Combined sewer overflow
CWS	Canadian Wildlife Service
DFO	Fisheries and Oceans Canada
DGGE	denaturing gradient gel electrophoresis
DNR	Department of Natural Resources (US)
DOM	Dissolved organic matter
EC	Environment Canada
EDC	Endocrine Disrupting Compound
EDS	Endocrine Disrupting Substance
EELS	electron energy loss spectroscopy
EPS	Environmental Protection Service
GFP	green fluorescent protein
GMO	Genetically Modified Organism
MISA	Municipal Industrial Strategy for Abatement
MITE	Metals in the Environment
MMT	Methylcyclopentadienyl manganese tricarbonyl
MOEE	Ministry of Environment and Energy
MTO	Ministry of Transport
NATO	North Atlantic Treaty Organisation
NPRI	National Pollutant Release Inventory
NSERC	Natural Sciences & Engineering Research Council of Canada
NWRI	National Water Research Institute
PAH	Polynuclear aromatic hydrocarbons
PNN	Probalistic neural network
SCLM	scanning confocal laser microscopy
SEM	scanning electron microscope

SETAC	Society of Environmental Toxicology and Chemistry
SMCCP	short and medium chain chlorinated paraffins
STAGE	Strategic Technologies Applications of Geonomics for the Environment program
STEM-EDS	Scanning Transmission Electron Microscopy – Energy Dispersive Spectroscopy
STP	sewage treatment plant
SUNY	State University of New York
TBT	tributyltin
TIE	Toxic Identification Evaluation
TP	total phosphorus
TSRI	Toxic Substances Research Initiative
UV	ultraviolet
YES	yeast estrogen system

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