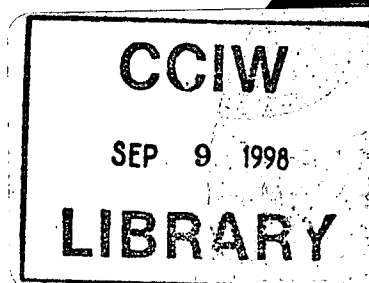


98-050

E1



**NATIONAL WATER
RESEARCH INSTITUTE**

**INSTITUT NATIONAL DE
RECHERCHE SUR LES EAUX**

**AQUATIC ECOSYSTEM PROTECTION BRANCH
CURRENT RESEARCH 1997-98**

Aquatic Ecosystem Protection Branch

NWRI Contribution No. 98-050

TD
226
N87
No. 98-
50

**AQUATIC ECOSYSTEM PROTECTION BRANCH
CURRENT RESEARCH
1997/98**

Aquatic Ecosystem Protection Branch
National Water Research Institute
Environment Canada
Canada Centre for Inland Waters
867 Lakeshore Rd, P.O. Box 5050
Burlington, Ontario
Canada L7R 4A6

NWRI Contribution No. 98-050

MANAGEMENT PERSPECTIVE

This report presents an overview of the research carried out in 1997-98 by the Aquatic Ecosystem Protection Branch of the National Water Research Institute. We hope that this document will provide useful information to our numerous national and international clients, visitors and collaborators.

R.J. Maguire
Acting Branch Director

SOMMAIRE À L'INTENTION DE LA DIRECTION

Ce document dresse un aperçu de la recherche effectuée en 1997-98 par la Direction de la protection des écosystèmes aquatiques de l'Institut national de recherche sur les eaux. Nous espérons que ce document sera utile à nos nombreux clients, visiteurs et partenaires canadiens et étrangers.

Le Directeur intérimaire de la Direction
R.J. Maguire

NWRI Contribution 98-050

To obtain more information, please contact
the Branch:

**Aquatic Ecosystem Protection Branch
National Water Research Institute
Environment Canada
Canada Centre for Inland Waters
867 Lakeshore Road, P.O. Box 5050
Burlington, Ontario L7R 4A6**

**Telephone: (905) 336-4928
Fax: (905) 336-4420**

Pour de plus amples informations, veuillez
contacter la Direction à l'adresse suivante:

**Direction de la protection des écosystèmes
aquatiques
Institut national de recherche sur les eaux
Environnement Canada
Centre canadien des eaux intérieures
867 Chemin Lakeshore, C.P. 5050
Burlington (Ontario) L7R 4A6
Téléphone: (905) 336-4928
Télécopieur: (905) 336-4420**

Aquatic Ecosystem Protection Branch - Current Research 1997/98

CONTENTS

| | |
|--|-----------|
| Biodiversity in Aquatic Ecosystems and Impacts of Municipal Effluents, <i>M. Allard</i> | 1 |
| Priority Substances: Pathways/Fate - <i>R.J. Maguire, Chief</i> | 5 |
| Fate of Pesticides and Industrial Chemicals in Water, <i>R.J. Maguire</i> | 7 |
| LC-GC-MS Identification of Toxic Substances, <i>D.T. Bennie</i> | 9 |
| Priority Polycyclic Aromatic Compounds in Energy Production and Use, <i>B.G. Brownlee</i> | 13 |
| Environmental Effects of Metals and Organometals, <i>Y.K. Chau and F. Yang</i> | 17 |
| Occurrences of Priority Organic Chemicals in Environmental Samples and Effluents, <i>H.-B. Lee</i> | 19 |
| Biodegradation of Priority and Targeted Chemicals, <i>D. Liu</i> | 23 |
| Sources and Fate of Toxic Substances, <i>F.I. Onuska</i> | 27 |
| Non-Point Sources of Pollution - <i>J. Marsalek, Chief</i> | 31 |
| Prevention and Remediation of Urban Non-Point Sources of Pollution, <i>J. Marsalek</i> | 33 |
| Aquatic Sediment Structure: Implications for Non-Point Source Sediment and Contaminant Transport, <i>I.G. Droppo</i> | 37 |
| Statistical Modelling of Environmental Changes, <i>A.H. El-Shaarawi</i> | 41 |
| Transport Characteristics of Fraser River Sediment and the Sediment/Contaminant/Biota Interactions, <i>B.G. Krishnappan</i> | 45 |
| Sediment/Contaminant Transport in Rivers, <i>Y.L. Lau</i> | 49 |
| Characterisation of Physically-Unstable Aquatic Colloids Relevant to Flocculation, <i>G.G. Leppard</i> | 53 |
| Agricultural Non-Point Sources of Pollution and Sustainable Agriculture, <i>H.Y.F. Ng</i> | 55 |
| Priority Substances - Exposure/Effects - <i>M.R. Servos, Chief</i> | 59 |
| Environmental Chemistry and Effects of Priority Substances, <i>M.R. Servos</i> | 61 |
| Reproductive Toxicology, Endocrinology, <i>S.B. Brown</i> | 67 |
| Xenoestrogenic Screening Procedure to Identify Substances of Concern in the Great Lakes Ecosystem, <i>B.K. Burnison</i> | 71 |
| Development and Application of Quantitative Structure-Activity Relationships to Acute, Chronic, Reproductive and Developmental Data/Endpoints, <i>K.L.E. Kaiser</i> | 73 |
| Fate and Effects of Contaminants on the Aquatic Ecosystems, <i>T. Mayer</i> | 77 |
| Impacts of Toxic Chemicals and Other Stresses on the Biodiversity of Freshwater Mussels in the Lower Great Lakes Drainage Basin, <i>J.L. Smith</i> | 79 |

CURRENT RESEARCH, FY 97/98

STUDY TITLE: Biodiversity in Aquatic Ecosystems and Impact of Municipal Effluents

STUDY LEADER: M. Allard

The review of the status and environmental impacts of municipal wastewater effluents on Canadian waters was completed. A study was initiated on the status of the biological diversity of aquatic invertebrates, with a special emphasis on the Laurentian Great Lakes. Highlights of these two activities are presented below:

(1) Review of the impact of municipal effluents on Canadian waters

Rivers, lakes and coastal waters have long been used as receptacles for diluting and dispersing domestic waste. While there have been considerable efforts since the 1970s by developed nations to improve sewage treatment, discharge of untreated or poorly treated sewage is still a concern in many parts of the world. In the case of developing countries, >90% of urban sewage is discharged directly into surface water without treatment. However, even in many developed countries, only a portion of municipal sewage receives conventional treatment. For example, only 60% of the total population of countries belonging to the Organisation for Economic Co-operation and Development was served by domestic wastewater treatment in the late 1980s.

The impacts of untreated or poorly treated domestic wastewater discharge on receiving water are numerous. Sewage also poses a direct human health risk from pathogens and indirect risk through exposure to microbiologically-contaminated waters during recreational activities or through consumption of contaminated shellfish. Potential environmental threats include excessive nutrient loading which can lead to eutrophication and subsequent effects on productivity and biodiversity; high loads of oxygen-demanding material which can reduce dissolved oxygen to levels that threaten the survival of aquatic organisms; contaminants which can cause immediate toxic effects on the biota and, in the case of persistent and bioaccumulative contaminants, can result in cumulative impacts on the receiving environment.

Canada is often seen as a privileged country because of the abundance and quality of its water resources. However, concerns raised by international agencies about sewage disposal world-wide and by public groups about the Canadian situation emphasized the need to evaluate the current status of municipal wastewater discharges on the Canadian environment. In September 1995, the Canadian Council of Ministers of the Environment (CCME) tasked an *ad hoc* working group reporting to the Environmental Protection Committee with exploring options to ensure environmental and human health protection from municipal wastewater effluents. In support of this initiative, a scientific review was initiated on the current risks to human health and the environment from municipal wastewater discharge in Canada.

Effective management of municipal wastewaters takes into consideration discharges not only from treatment plants, but also from stormwater and combined sewer overflows (CSO). Discharges from municipal wastewaters have caused some adverse impacts on lakes, rivers and coastal waters. The

environmental consequences of municipal wastewater discharges to the Canadian environment are difficult to generalize, largely due to regional variations in level of sewage treatment plant and in the nature of the receiving environment. The most publicly recognized impacts are shellfish harvesting restrictions and beach closures resulting from microbial contamination. Contamination of shellfish harvesting beds is widespread and pervasive on both the Atlantic and Pacific coast and resulted in significant economic loss. Habitat degradation and contamination also occur and these, in turn, have altered the abundance and diversity of aquatic organisms. Destruction of fish and wildlife habitats caused by stormwater and CSO discharges have been documented. Further research is also required on the interactive and cumulative responses to long-term chemical exposure, including the potential risk associated with endocrine disrupting substances, and to habitat degradation. Finally, an integrated approach to wastewater management is needed that addresses loadings from treatment plants, stormwater sewers, combined sewer overflows and other wastewater sources.

(2) Biodiversity of aquatic invertebrates

The United Nations Convention on Biological Diversity (CBD) entered into force in 1993. The CBD encourages its Parties, of which Canada is a member, to take action within the context of three general goals enshrined in the convention, i.e, the conservation of biodiversity, the sustainable use of biological resources and the fair and equitable sharing of benefits arising from the use of genetic diversity. In response to the CBD, Canada has published its Canadian Biodiversity Strategy in 1995.

Since 1993, The Conference of the Parties (COP) to the CBD has focused its attention on the status of biodiversity in various ecosystems. For the first time, the COP at their 1998 meeting will be examining the status of biodiversity in inland waters (mainly fresh water). On the global scale, the biodiversity of freshwater ecosystems has received much less attention than other types of ecosystem. Although freshwater biodiversity is among the most poorly known on earth, it has been reported by many to be seriously threatened given the status of the world's freshwater ecosystems. Very few organisms, other than mammals or fish, are monitored or considered. Much of what we now know is based on our, often incomplete, knowledge of fish species. Biodiversity losses are presumed to be just as bad, if not worse, for aquatic invertebrates. However, we know very little of the vast majority of them.

Our study was initiated in order to take a closer look at the current status and initiatives concerning biodiversity in aquatic ecosystems at the international, North American and Canadian levels. The report in preparation briefly discusses the current status of and threats to biodiversity in aquatic ecosystems; examines, within a biodiversity context, past and current work on aquatic invertebrates in the Great Lakes basin; outlines knowledge gaps, difficulties and opportunities; discusses promising initiatives and actions to achieve progress on our CBD obligations, within the context of the Canadian Biodiversity Strategy.

Articles/reports/conference presentations in FY 97/98

- (1) Allard, M., Q. Rochfort, J. Marsalek, B.J. Dutka and J. Shaw. Exploratory study of toxicity of urban stormwater. *Presented at the 9th World Water Congress, Montréal, 1-6 September 1997.*
- (2) Chambers, P.A., M. Allard, S.L. Walker, J. Marsalek, J. Lawrence, M. Servos, J. Busnarda, K.S. Munger, K. Adare, C. Jefferson, R.A. Kent and M.P. Wong. 1997. The impacts of municipal wastewater effluents on Canadian waters: A review. *Water Qual. Res. J. Canada* 32(4): 659-713.

CURRENT RESEARCH, FY 97/98

PRIORITY SUBSTANCES - PATHWAYS/FATE PROJECT AQUATIC ECOSYSTEM PROTECTION BRANCH

The Project carries out research on fundamental mechanisms governing the persistence and fate of toxic chemicals in aquatic environments. This knowledge is used by Environment Canada to assess the hazards posed by toxic chemicals released to the aquatic environment. The focus is on the provision of appropriate scientific information to support decision-making for the Toxic Substances Management Policy (TSMP), the Canadian Environmental Protection Act (CEPA), the Pest Control Products Act (PCPA), regional ecosystems programs and ecosystems indicators. Following is a summary of study highlights for fiscal year 1997/1998.

CURRENT RESEARCH, FY 97/98

PROJECT: Priority Substances - Pathways/Fate
STUDY TITLE: Fate of Pesticides and Industrial Chemicals in Water
STUDY LEADER: R.J. Maguire

Research was completed on the toxicity of sediment-bound tributyltin to benthic organisms (see below). Research continues on the persistence of tributyltin in sediment cores, on the toxicity of sulfonylurea herbicides to aquatic plants, and on the occurrence of certain priority chemicals in industrial areas (nonylphenol and ethoxylates, chlorinated paraffins, trialkyl/aryl phosphates), in collaboration with other study leaders. Major activities are summarized in other Project study leaders' Current Research contributions.

(1) Toxicity of tributyltin to benthic organisms

The toxicity of tributyltin (TBT) in sediment in freshwater was determined for four benthic invertebrates, (the oligochaete worm *Tubifex tubifex*, the chironomid *Chironomus riparius*, the amphipod *Hyaella azteca*, and the mayfly *Hexagenia*). Although there was some degradation of spiked TBT into dibutyltin (DBT) and monobutyltin (MBT) in the sediment-water mixtures during the acclimation phase before toxicity testing, the contribution of these degradation products to the observed toxicity was assumed to be minimal based on evidence that TBT is up to orders of magnitude more toxic to many aquatic organisms than its degradation products. The results of this work indicated that *Hexagenia* was the most sensitive benthic organism to TBT of the four organisms tested, with an IC_{50} value (growth) for TBT of $0.6 \mu\text{g Sn/g dry weight}$. This value is about six times greater than mean concentrations of TBT observed in sediment in fresh water in surveys conducted across Canada before and after the regulation of antifouling uses of TBT in 1989. However, maximum TBT concentrations in sediment in both periods exceeded the IC_{50} value (growth) for *Hexagenia*, and were close to the lower limits of toxicity ranges for *H. azteca* and *T. tubifex* (cocoons/adult and young/adult). Consequently, at the time of sample collection there was potential for adverse effects of TBT in some sediments to fresh water benthic organisms.

Articles/reports/conference presentations in FY 97/98

- (1) Day, K.E., R.J. Maguire, D. Milani and S.P. Batchelor. 1998. Toxicity of tributyltin to four species of freshwater benthic invertebrates using spiked sediment bioassays. *Water Qual. Res. J. Canada* 33: 111-132.
- (2) Thompson, J.A.J., S. Douglas, Y.K. Chau and R.J. Maguire. Recent studies of residual tributyltin in coastal British Columbia sediments. *Appl. Organometal. Chem.*, *in press*.

- (3) Bennie, D.T., C.A. Sullivan, H.-B. Lee and R.J. Maguire. A preliminary study of the occurrence of alkylphenol polyethoxylate surfactant metabolites in Canadian sewage treatment plants. *(submitted)*
- (4) Yang, F., Y.K. Chau and R.J. Maguire. Occurrence of butyltin compounds in beluga whales (*Delphinapterus leucas*). *Appl. Organometal. Chem.*, *in press*.
- (5) Liu, D., G.J. Pacepavicius, R.J. Maguire, Y.L. Lau, H. Okamura and I. Aoyama. Mercuric chloride - catalyzed hydrolysis of the new antifouling compound Irgarol 1051. *Water Res.*, *in press*.
- (6) Lee, H.-B., J. Weng, T.E. Peart and R.J. Maguire. 1998. Occurrence of alkylphenoxyacetic acids in Canadian sewage treatment plant effluents. *Water Qual. Res. J. Can.* **33**: 19-29.
- (7) Lee, H.-B., T.E. Peart, S.P. Batchelor, J. Weng and R.J. Maguire. 1997. Liquid and gas chromatographic determination of sulfonylurea herbicides metsulfuron methyl and ethametsulfuron methyl in water and soil samples. NWRI Contribution 97-097.
- (8) Sullivan, C.A., D.T. Bennie and R.J. Maguire. 1997. Occurrence of aniline in Canagagigue Creek, Ontario - 1995-1996. AEPB Technical Report TN97-004.
- (9) Onuska, F.I., K.A. Terry and R.J. Maguire. 1997. High resolution gas chromatography - mass spectrometry of some benzidines in industrial waste water. NWRI Contribution 97-202.
- (10) Allan, L.M., D.K. Verma, F. Yang, Y.K. Chau and R.J. Maguire. 1998. Evaluation of extraction procedures for the analysis of butyltin compounds in air. NWRI Contribution 98-030.
- (11) Maguire, R.J. An appreciation of Y.K. Chau. *Appl. Organometal. Chem.*, *in press*.
- (12) Liu, D., G.J. Pacepavicius, R.J. Maguire, Y.L. Lau, H. Okamura and I. Aoyama. Review of cyclone fermentor test for assessing the biodegradability of toxic substances. *Presented at the 33rd Central Canadian Symposium on Water Pollution Research*, Burlington, Ontario, Feb. 9, 1998.
- (13) Maguire, R.J. Environmental assessment of organotin compounds in Canada. *Presented at the European Union "Speciation 21" Workshop on Environmental Issues - 1. Assessing the Impact of Organotin Compounds in the Environment*. Segovia, Spain, March 16-17, 1998.
- (14) Maguire, R.J.. Assessment of the tributyltin situation in Canada. *Presented at the 215th American Chemical Society National Meeting*, Dallas, TX, U.S.A., March 29 - April 2, 1998, Division of Environmental Chemistry 38 (1), 124-126.

CURRENT RESEARCH, FY 97/98

PROJECT: Priority Substances - Pathways/Fate

STUDY TITLE: LC-GC-MS Identification of Toxic Substances

STUDY LEADER: D.T. Bennie

Research continues in the following areas: the occurrence of nonylphenol and nonylphenol polyethoxylates in natural waters, textile mill effluent and municipal sewage treatment plant effluent; and the biological effects of alkylphenol and PAH-contaminated water and sediments on caged rainbow trout in Hamilton Harbour. Experimental aspects of studies dealing with the persistence of nonylphenolic substances in biosolids-treated agricultural soil and the occurrence of short-chain chlorinated paraffins in biological specimens, such as Hamilton Harbour carp, rainbow trout from Lake Ontario and beluga from the St. Lawrence River, have been completed. The major activities of each study are reported below:

(1) The occurrence of nonylphenol and nonylphenol polyethoxylates in natural waters, textile mill effluent and municipal sewage treatment plant effluent

Research 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 and textile mills. Three sewage treatment plants (STPs) were sampled on a monthly basis, including a state-of-the-art tertiary plant that was also studied comprehensively for a two-week period. The river into which the tertiary plant discharges was also surveyed during the same two-week period. Results from three Atlantic Region textile mills, two of which discharge to municipal sewage treatment plants, were also obtained.

During the two-week study a variety of alkylphenolic parameters were determined as well as the conventional STP operational parameters. The alkylphenolic parameters were 4-nonylphenol (4-NP), nonylphenol ethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO), nonylphenoxycetic acid (NP1EC), nonylphenoxymethoxycetic acid (NP2EC), nonylphenol polyethoxylates (NPnEO, where n = 3 to 17), 4-*tert*-octylphenol (4-*t*-OP), octylphenoxycetic acid (OP1EC) and octylphenoxymethoxycetic acid (OP2EC). The sum of these parameters is referred to as total alkylphenolics (T-AP). Over the studied period, the T-AP concentrations in the raw sewage ranged from 489 µg/L to 3,027 µg/L, and the lowest values were measured after the weekend when the plant has no textile mill input. The raw sewage was composed mainly of nonylphenol ethoxylate compounds (NP1EO, NP2EO & NPnEO) (96.1% or 1380 µg/L) while alkylphenol carboxylate compounds made up 2.6% (38 µg/L) of the input and 4-NP accounted for 1.2% (17 µg/L) of the alkylphenolics coming into the plant. Effluent discharged into the river was composed of 4-NP at 3.6% (1.3 µg/L), total nonylphenol ethoxylates at 21.1% (7.8 µg/L), nonylphenoxycetic acid at 20.3% (7.5 µg/L) and nonylphenoxymethoxycetic acid at 54% (20 µg/L). Concentrations found in the digested sludge did not have the same alkylphenolic characteristics as other authors have suggested. Nonylphenol ethoxylate comprised 48.8% (562 µg/g) of the T-AP in the sludge, while NPnEO accounted for 25.9% (298 µg/g), 4-NP accounted for 13%

(150 µg/g) and NP2EO accounted for 10.8% (124 µg/g). The levels of these estrogenic compounds in the digested sludge may be cause for concern with respect to the disposal of the sludge.

Preliminary results for the river samples into which the above-mentioned facility discharges suggest that 4-NP results are elevated at the point of discharge (about 1.1 µg/L - comparable to the final effluent values obtained in the 2 week study) and the effluent is completely mixed with the river water by 4 km downstream and 4-NP values have decreased to background levels. Background levels of 4-NP in this river are about 0.15 µg/L.

Textile mills are significant sources of alkylphenolic substances and nonylphenol polyethoxylates, in particular. The tertiary sewage treatment mentioned previously was chosen for that study because it receives significant inputs from three textile processing mills. In Atlantic Canada, three textile mills' final effluent streams have been sampled and analyzed for 4-NP, NP1EO, NP2EO, NPnEO and 4-*t*-OP. Two of these mills discharge into municipal STPs and their raw and final effluents were analyzed as well. Final effluents from the textile mills were, predictably, heavily loaded with ethoxylated nonylphenolics. Results ranged from 50 µg/L to 8600 µg/L in mill effluents for NPnEO, with the lowest values coming from a plant that utilizes small batch processing. High values were associated with plants that run continuously, 24 hours a day for the five-day work week. Concentrations of 4-NP in final mill effluents ranged from 1.1 µg/L to 13.3 µg/L. Both STPs were primary treatment facilities only, but 4-NP levels were diminished substantially, with results in the STP final effluents ranging from 1.4 µg/L to 2.0 µg/L. Nonylphenol polyethoxylate concentrations in STP final effluents ranged from 14 µg/L to 400 µg/L.

(2) The biological effects of alkylphenol and PAH- contaminated water and sediments on caged rainbow trout in Hamilton Harbour

In collaboration with scientists from the University of Guelph, the University of Waterloo and NWRI, a study was undertaken to ascertain the biological effects of alkylphenol and PAH-contaminated water and sediments on caged rainbow trout in Hamilton Harbour. Our role in this study was to determine concentrations of 4-NP and the EPA priority PAHs in the surface water of the six sampling sites, the sediment and the fish tissue. Values of 4-NP in the surface water ranged from 0.06 µg/L at the control site in Lake Ontario to 1.0 µg/L at the Hamilton Harbour Windemere Basin site. Fish tissue concentrations ranged from not detectable (< 0.010 µg/g) in specimens caged at the control site to 0.07 µg/g in fish caged at another Windemere Basin site near a large steel plant on Hamilton Harbour. Analyses for PAHs are still in progress and will be reported at a later date.

(3) Persistence of nonylphenolic substances in biosolids-treated agricultural soil

A small study was conducted to determine the persistence of alkylphenol polyethoxylate surfactants and their metabolites in wastewater biosolids (digested sludge) that are used as an additive to agricultural land. Dewatered digested sludge from a tertiary STP was applied to the soil of four experimental plots at the University of Guelph, Elora Research Station. Sludge was applied at a rate of 0, 8 (the legal application rate in Ontario), 20 and 40 tonnes per hectare (t/ha) of dry soil and tilled into the soil. Sludge-amended soil samples were collected at 0, 1, 3, 6, 10, 15, 24, 41, 62, 90 and 121 days after application. The dewatered digested sludge was also characterized before application.

Concentrations of NPnEO, NP2EO and NP1EO in the dewatered sludge were about 350 mg/kg, 10 mg/kg and not detectable, respectively. Concentrations of these substances in the untreated and treated soil samples were consistently below the minimum detection limits throughout the study period. The dewatered digested sludge contained about 12 mg/kg of alkylphenol carboxylic acid metabolites. When diluted by application to the agricultural soil, these substances were detected throughout the experimental period but the results were below the quantifiable detection limits of 0.5 mg/kg for NP1EC and NP2EC and 0.05 mg/kg for OP1EC and OP2EC.

The concentration of 4-NP and 4-*t*-OP in the digested sludge were found to be about 450000 µg/kg and 15000 µg/kg, respectively. In the 8 t/ha treated soil plot, the concentrations of these substances remained approximately constant for the first 15 days and decreased gradually over the study period to below the minimum detection limits by about day 90. The plots with higher application rates had detectable levels of 4-NP and detectable but not quantifiable levels of 4-*t*-OP throughout the whole period.

(4) The occurrence of short-chain chlorinated paraffins or polychlorinated alkanes (PCAs) in biological specimens, such as Hamilton Harbour carp, rainbow trout from Lake Ontario, and beluga from the St. Lawrence River

Beluga fat and liver tissue, received from Dr. R. Bailey (Maurice Lamontagne Institute, Fisheries and Oceans Canada) were analyzed by low resolution negative chemical ionization gas chromatography-mass spectrometry (NCI GC-MS). Concentrations of PCAs in beluga liver tissue ranged from 0.24 to 5.7 mg/kg wet weight. However, concentrations in beluga fat from the same animals were substantially higher with values ranging from 1.4 to 200 mg/kg wet weight. Fat collected at different depths on the beluga carcasses did not show any appreciable differences in PCA concentration.

Rainbow trout collected in western Lake Ontario by staff from the Great Lakes Laboratory for Fisheries and Aquatic Sciences (Fisheries and Oceans Canada) had PCA concentrations ranging from 0.45 to 3.2 mg/kg wet weight while carp collected in Hamilton Harbour had concentrations ranging from 0.03 to 0.38 mg/kg wet weight. Trout and carp samples were homogenized whole fish tissue samples.

Numerous sediment samples from Hamilton Harbour, Toronto Harbour, Montréal Harbour, the mouth of the Credit River and the mouth of the Humber River were screened by GC-ECD for PCAs and the results were negative.

Results of these analyses will be compared to results obtained by high resolution NCI GC-MS analysis in collaboration with Dr. Derek Muir (NWRI) and Dr. Gregg Tomy (Fisheries and Oceans Canada).

Articles/reports/conference presentations in FY 97/98

- (1) Bennie, D.T., C.A. Sullivan, H.-B. Lee, R.J. Maguire. 1998. Alkylphenol polyethoxylate metabolites in Canadian sewage treatment plant waste streams. *Water Qual. Res. J. Canada* **33**(2):231-252.

- (2) Sullivan, C.A., D.T. Bennie and R.J. Maguire. 1997. Occurrence of aniline in Canagagigue Creek, Ontario - 1995-1996. NWRI Technical Report AEPB-TN-97-004.
- (3) Bennie, D.T., C.A. Sullivan, H.-B. Lee, R.J. Maguire. Alkylphenol polyethoxylate metabolites in Canadian sewage treatment plant waste streams. *Presented at the 19th Annual Meeting of the Society of Environmental Toxicology and Chemistry, San Francisco, CA, USA, November 16-20, 1997.*

CURRENT RESEARCH, FY 97/98

PROJECT: Priority Substances - Pathways/Fate
STUDY TITLE: Priority Polycyclic Aromatic Compounds in Energy Production and Use
STUDY LEADER: B.G. Brownlee

Research was carried out on polycyclic aromatic compounds (PACs) in oil sands fine tailings and in urban runoff. PACs are of concern because they belong to a group of compounds which contains many mutagenic or carcinogenic compounds. Part of this PAC research has been to continually expand the range of compounds analyzed in water and solids. This also addresses a high priority research recommendation of the CEPA PSL1 assessment of polycyclic aromatic hydrocarbons (PAHs). Collaborative research on the analysis and identification of taste and odour compounds was continued with J. Ridal of the St. Lawrence River Institute of Environmental Sciences and S. Watson and E. McCauley of the University of Calgary. Research activities in these areas are summarized below.

(1) Polycyclic aromatic compounds in oil sands fine tailings

Extraction of bitumen from oil sands by the Clark hot water process generates large quantities of fine tailings which consist of fine sand, silt, clay, water, and unrecovered bitumen. One of the groups of compounds present in the fine tailings and of concern for site reclamation are the PACs. Fine tailings were separated by high speed centrifugation (ca. 30,000 x g) into the solids containing "bound" PACs, and into free water (porewater) containing "dissolved" PACs. A multi-step procedure was worked out to give a complete separation of the acid and base/neutral fractions extracted from porewater by dichloromethane (DCM). The base/neutral fraction contains the PACs. Several litres of porewater were processed by this procedure to provide fractions for qualitative analysis and toxicity testing. The base/neutral fraction was further subjected to open column chromatography and to semi-preparative high performance liquid chromatography (HPLC) followed by analysis by gas chromatography-mass spectrometry on quadropole and ion trap mass spectrometers.

Concentrations of individual PACs and alkyl-PACs were all less than 1000 ng/L in porewater. The following parent PACs were specifically identified: acenaphthene, fluorene, dibenzothiophene, phenanthrene, fluoranthene and pyrene. The predominant alkyl-PACs were the alkylnaphthalenes, alkyldibenzothiophenes and alkylphenanthrenes.

As a screening test, base/neutral and acid extracts were tested by the MutatoxTM method at ca. 2000 times the concentration present in the original porewater. Both extracts were positive without metabolic activation and negative with metabolic activation (S9). Most of the model compounds tested were more active without metabolic activation. Correlation with literature results for the widely used Ames *Salmonella* test was only 45%. Since the MutatoxTM test results were somewhat ambiguous, further testing by the Ames test was carried out.

Testing of base/neutral and acid extracts at ca. 10,000 times the concentration in the original porewater gave no evidence of mutagenicity with or without metabolic activation using *Salmonella typhimurium* strains TA98 and TA100.

(2) Polycyclic aromatic compounds in urban runoff

Urban runoff samples from a variety of sources (road runoff, combined sewer overflows) have been analyzed for a wide range of PACs (primarily polycyclic aromatic hydrocarbons and alkyl-PAHs). The samples are first filtered to separate the water and particulate fractions which are extracted and analyzed to give dissolved and particulate concentrations of PACs.

Two refinements to our analytical procedures were introduced. (a) A cleanup method using normal phase semi-preparative HPLC to remove less polar material (unresolved complex mixture, UCM) which was interfering with quantitation of the chromatograms. (b) Accelerated solvent extraction (ASE) as an alternative to Soxhlet extraction of the particulates. ASE compares well with Soxhlet extraction for extraction efficiency and reduces solvent use by a factor of 5-10.

Earlier work on toxicity testing of urban runoff samples has been reported (Rochfort et al. 1998).

(3) Taste and odour compounds in water supplies

In collaboration with J. Ridal of the St. Lawrence Institute of Environmental Sciences, a study was carried out to measure two taste and odour compounds, geosmin and 2-methylisoborneol, in the Cornwall Water Treatment Plant and the use of newly installed granular activated carbon filters for their reduction. Earlier results on these two compounds in the upper St. Lawrence River were presented at conferences (Ridal et al. 1977) and a manuscript is in preparation.

In a collaborative study with S. Watson and E. McCauley of the University of Calgary, the recently developed technique of solid phase microextraction (SPME) was applied to the analysis of odorous unsaturated aldehydes produced by chrysophytes (e.g., *Dinobryon* sp., *Symura* sp. and *Uroglena* sp.) in cultures and ponds. These algal genera have been implicated in periodic springtime taste and odour events in the Glenmore Reservoir which serves as one of the sources of raw water for the City of Calgary. We were able to tentatively identify 2*t*,4*c*-heptadienal in *Uroglena* cultures and pond water. The detection limit for the method is currently about 1-2 µg/L which will have to be lowered if the method is to be applied successfully to the analysis of reservoir samples. These results were presented at a major international symposium and have been submitted for publication (Watson et al. 1998). A manuscript on the methodology is in preparation.

Articles/reports/conference presentations in FY 97/98

- (1) Ridal, J.J., B. Brownlee, J. Carney and D.R.S. Lean. Taste and odour compounds in upper St. Lawrence River drinking water. *Presented at the 4th Annual Symposium on the St. Lawrence River Ecosystem*, Potsdam, NY, April 15-17, 1997.
- (2) Ridal, J.J., B. Brownlee and D.R.S. Lean. An overview of taste and odour problems in drinking water. *Presented at the fall meeting of the Southeastern Water Association of Ontario*, November 26, 1997.
- (3) Rochfort, Q., J. Marsalek, B. Dutka, B. Brownlee, A. Jurkovic, R. McInnis and G. MacInnis. 1998. Acute toxicity of combined sewer overflows and stormwater discharges. NWRI Contribution No. 97-190.

- (4) Watson, S.B., B. Brownlee, T. Satchwill and E. McCauley. The use of solid phase microextraction (SPME) to monitor for major organoleptic compounds produced by chrysophytes in surface waters. *Presented at the Fifth International Symposium on Off-Flavours in the Aquatic Environment*, Paris, France, October 13-16, 1997. Also submitted to *Water Science and Technology*.

CURRENT RESEARCH, FY 97/98

PROJECT: Priority Substances - Pathways/Fate
STUDY TITLE: Environmental Effects of Metals and Organometals
STUDY LEADER: Y.K. Chau and F. Yang

Research was completed on the determination of butyltin compounds in Beluga whale tissues, sediment cores and air samples, and the development of analytical technique for the gasoline additives MMT (methylcyclopentadienylmanganese tricarbonyl) and MTBE (methyl *tert*-butyl ether) in aqueous samples. Major studies are summarized as follows:

(1) Occurrence of organotin compounds in Beluga whale in the St. Lawrence River

Antifouling uses of tributyltin (TBT) have caused great environmental concern because of its high toxicity to nontarget marine biota. In this study, Beluga samples (6 liver and 46 blubber samples) from the St. Lawrence River, Canada, were analyzed for butyltin compounds to investigate the occurrence and contamination of butyltin in these marine mammals. A special procedure was developed for the determination of butyltin species with high lipid content (up to 95%). Total butyltin concentrations in liver samples were found to be much higher than those in the blubber samples, and the concentration levels of butyltin species in blubber samples were observed to be related to their lipid content.

(2) Determination of MMT and MTBE in aqueous samples with SPME method

Both MMT and MTBE are used as octane boosters in unleaded gasoline. MMT is toxic and emission of manganese to the atmosphere may cause chronic manganese poisoning, leading to Parkinsonism-like effects. MTBE is a known irritant and frequently detected in shallow groundwater from urban areas. SPME (solid phase microextraction) is a simple, efficient and solvent-free sampling technique. A method was developed to extract MMT and MTBE in headspace over water sample with a poly(dimethylsiloxane)-coated fused silica fiber. The detection limits are 0.3 pg/L as Mn for MMT and 100 ng/L for MTBE with the combination of SPME technique and the highly sensitive and selective GC-AED. MMT was detected in highway run-off and sewage samples by this method.

(3) Determination of butyltin compounds in sediment cores

TBT is extremely toxic to certain aquatic organisms. Consequently, its use as an antifoulant was regulated in 1989 under the Pest Control Products Act in Canada. We were interested in determining the persistence of TBT in sediment cores taken from harbours across Canada. The cores were sliced into 1 cm sections which were dated by lead-210 and analyzed for the concentrations of butyltin species. The accumulation and degradation of butyltin compounds were observed from the distribution profiles of TBT and its degradation products, DBT and

MBT, in sediment cores. In sediment, the butyltin compounds are persistent and their concentrations decrease gradually after the antifouling use of butyltin compounds was regulated in Canada.

(4) Collection and analysis of butyltin compounds in air

Although the toxicity of organotin compounds has raised public health concerns, there have been few investigations on the existence of butyltin compounds in air because of their low volatilities. An analytical method for determining butyltin chlorides in ambient atmosphere was developed in our laboratory in collaboration with a research group from McMaster University that was interested in air sampling at an industry that used butyltins in glass coating. The butyltin species were collected on glass fiber filters followed by an XAD-2 sorbent tube, extracted with sodium diethyldithiocarbamate (NaDDC) in toluene, and then derivatized with pentyl Grignard reagent. After the clean-up with silica gel column, the extract was analyzed by GC-AED. Recoveries of butyltin and triphenyltin compounds ranged from 81 to 91%.

Articles/reports/conference presentations in FY 97/98

- (1) Yang, F., Y.K. Chau and R.J. Maguire. 1998. Occurrence of butyltin compounds in Beluga whales (*Delphinapterus leucas*). Appl. Organomet. Chem. *In press*.
- (2) Yang, F., X.C. Fang, M. Alaei and Y.K. Chau. 1998. Solid phase extraction of butyltin compounds in aqueous samples following derivatization *in situ* with sodium tetraethylborate. NWRI Contribution No. 98-022.
- (3) Chau, Y.K. and F. Yang. 1997. A novel technique for direct derivatization of ionic organotin and alkyl-lead compounds in sediment: Simultaneous determination of organotin and alkyl-lead compounds in sediment by gas chromatography-plasma atomic emission (GC-AED) dual channel detection. Appl. Organomet. Chem. **11**: 851-858.
- (4) Chau, Y.K., R.J. Maguire, M. Brown, F. Yang, S.P. Batchelor and J.A.J. Thompson. 1997. Occurrence of butyltin compounds in mussels in Canada. Appl. Organomet. Chem. **11**: 903-912.
- (5) Chau, Y.K., F. Yang and M. Brown. 1997. Evaluation of derivatization techniques for the analysis of organotin compounds in biological tissue. Anal. Chim. Acta. **338**: 51-55.
- (6) Chau, Y.K., F. Yang and M. Brown. 1997. Determination of methylcyclopentadienyl-manganese tricarbonyl (MMT) in gasoline and environmental samples by gas chromatography with helium microwave plasma atomic emission detection. Appl. Organomet. Chem. **11**: 31-37.
- (7) Chau, Y.K., R.J. Maguire, M. Brown, F. Yang and S.P. Batchelor. 1997. Occurrence of organotin compounds in the Canadian aquatic environment five years after the regulation of antifouling uses of tributyltin. Water Qual. Res. J. Canada **2**: 453-521.

CURRENT RESEARCH, FY 97/98

PROJECT: Priority Substances - Pathways/Fate
STUDY TITLE: Occurrence of Priority Organic Chemicals in Environmental Samples and Effluents
STUDY LEADER: H.-B. Lee

Development of methods for the determination of estrogenic compounds in raw sewage and effluent using GC/MS techniques has been completed. A brief survey of 17 β -estradiol and its metabolites in Canadian sewage was also carried out. A twelve-month monitoring program for nonylphenolic compounds in the influent, primary and final effluents of a sewage treatment plant has also been completed. The analytical methods for nonylphenolic compounds have been reviewed. A survey on the occurrence of nonylphenol ethoxylates and their degradation products in pulp and paper mill effluents has been initiated. Major findings are listed below:

(1) Methodologies for estrogens

The syntheses of acetyl, pentafluoropropionyl, heptafluorobutyryl, and trimethylsilyl derivatives of estrogenic compounds such as diethylstilbestrol, 17 β -estradiol (E₂), estrone (E₁), estriol (E₃), and 17 α -ethynylestradiol (EE₂) has been attempted. These reactions were quantitative and the products were all stable at -20°C for weeks. Their separation on a HP-5 column was demonstrated. The GC/MS data indicated that, with the exception of EE₂, all hydroxy groups of these estrogens were derivatized. In the case of EE₂, the hydroxy group at the 17-position did not react. Intense molecular ions were observed, in many cases, for the pentafluoropropionyl and trimethylsilyl derivatives. Using these characteristic ions, detection of pg amounts of these estrogen derivatives could be achieved by GC/MS in selected ion monitoring mode. A report describing the preparation and GC/MS characteristic of these estrogen derivatives has been completed.

A simple and quantitative method designed for the monitoring of 17 β -estradiol (E₂) and its metabolites estrone (E₁) and estriol (E₃) as environmental contaminants in municipal sewage effluents has been developed. Preconcentration and cleanup of the estrogens were performed by a solid phase extraction technique using a reversed phase, C₁₈ cartridge. The estrogens were derivatized with pentafluoropropionic acid anhydride and the products were analyzed by gas chromatography-mass spectrometry. Recoveries of the analytes from spiked distilled water and sewage were better than 87% at fortification levels of 100 and 20 ng/L. With a concentration factor of 5000, the detection limits were 5 ng/L for E₁ and E₂ and 10 ng/L for E₃ in sewage samples. In a brief survey of Canadian wastewater, these estrogens were detected in many raw sewage and effluents samples at ng/L levels. A final report for this method has been prepared.

(2) Occurrence of nonylphenol ethoxylates and their metabolites in sewage samples

A twelve-month sampling and monitoring program for the occurrence of nonylphenolic compounds in the influent as well as the primary and final effluents of the Halton Skyway sewage treatment plant has been completed. Nonylphenol ethoxylates (NPEO) and their metabolites nonylphenol (NP) and carboxylates (NPEC) were readily detected in every sewage sample. Extensive degradation of NPEO in the raw sewage was evidenced by the observation of high levels of NP and the presence of NPEC in those samples. The sewage treatment processes were very effective in the removal of NPEO since there was an over 90% decrease in the levels of these compounds between the primary and final effluents. Elimination of NP was less effective and elevated levels of NPEC were observed in the final effluent. A final report is being prepared.

(3) Nonylphenol methodologies

A review of the major analytical methods published in the last 20 years for alkylphenol ethoxylates and their degradation products in environmental samples has been completed. In this review, traditional and modern extraction and preconcentration methods for water and solid samples were summarized. Chromatographic separation of these chemicals by normal and reversed phase HPLC as well as GC were compared. Various sensitive and selective detection techniques including the use of mass spectrometers were discussed.

(4) A survey of nonylphenolic compounds in paper mill effluent and sludge

A pilot study on the occurrence of nonylphenolic compounds in pulp and paper mill effluents and sludge has been initiated. Primary and secondary effluent samples provided by Avenor and PAPRICAN were analyzed for NPEO and NP. While these compounds were detectable, their levels, except for a few cases, were usually low (<50 µg/L for NPEO and <1 µg/L for NP). Sludge and biosolid samples provided by PAPRICAN contained <50 µg/g of NPEO and <1 µg/g of NP in most cases. This study addresses a data gap in the CEPA PSL2 assessment of nonylphenol, and will be continued in FY 98/99.

Articles/reports/conference presentations in FY 97/98

- (1) Lee, H.-B., J. Weng, T.E. Peart and R.J. Maguire. 1998. Occurrence of alkylphenoxyacetic acids in Canadian sewage treatment plant effluents. *Water Qual. Res. J. Canada* **33**: 19-29.
- (2) Bennie, D.T., C.A. Sullivan, H.-B. Lee and R.J. Maguire. Alkylphenol polyethoxylate metabolites in Canadian sewage treatment plants. *Water Qual. Res. J. Canada*, *in press*
- (3) Lee, H.-B. and T.E. Peart 1997. Derivatives of estrogens for gas chromatography-mass spectrometry analysis. NWRI Contribution No. 97-207.
- (4) Lee, H.-B. and T.E. Peart. 1998. Determination of 17β-estradiol and its metabolites in sewage effluent. NWRI Contribution No. 98-014.

- (5) Lee, H.-B. A review of analytical methods for the determination of nonylphenol and related compounds in environmental samples (*in preparation*)
- (6) Dutka, B.J., D. Liu, A. Jurkovic, R. McInnis, H.-B. Lee, F. Onuska and S.S. Rao. 1997. Observations from a six month study on the effect of biodegradation processes in sediment on toxicity potential of targeted chemicals. NWRI Contribution No. 97-208.

CURRENT RESEARCH, FY 97/98

PROJECT: Priority Substances - Pathways/Fate
STUDY TITLE: Biodegradation of Priority and Targeted Chemicals
STUDY LEADER: D. Liu

Research was completed on the abiotic degradation of the new antifouling compound Irgarol 1051 by mercuric chloride. A survey of Irgarol 1051 in Canadian and Japanese water and sediment is still in progress. Major activities are summarized below:

(1) Abiotic degradation of Irgarol 1051

The aim of this study was to elucidate the mechanism involved in the mercuric chloride-catalyzed hydrolysis of the new antifouling compound Irgarol 1051. This study showed that mercuric chloride was capable of rapidly catalyzing the hydrolysis of Irgarol 1051 in distilled water and buffer solutions. The degradation appeared to follow the reaction of a catalyzed hydrolysis, and was not significantly affected by pH (i.e., at pH 5 to 9). All the five other heavy metal salts tested (AgNO_3 , CdCl_2 , CuSO_4 , PbCl_2 , and ZnCl_2) had practically no catalytic effect on Irgarol hydrolysis, implying the involvement of a specific activity for Hg^{2+} in this reaction. The mechanism for the catalyzed hydrolysis appeared to involve the formation of bidentate chelation through nitrogen (No. 5) on the ring and the nitrogen on the cyclopropylamino side chain in Irgarol 1051 with the Hg^{2+} ion. The resulting four-member chelate complex would weaken the cyclopropyl-amino bond considerably, thus facilitating the hydrolysis reaction. Ultraviolet spectroscopy of the reaction mixtures and the identification of Irgarol hydrolysis product M1 (2-methylthio-4-*tert*-butylamino-6-amino-*s*-triazine) by GC-MS and LC-MS provided the basis for the proposed mechanism for the HgCl_2 -catalyzed hydrolysis of Irgarol 1051. M1 appeared to be more stable than the parent compound Irgarol 1051, thus implying its possible accumulation in the environment. One practical aspect of this work is that HgCl_2 should not be used in preserving water samples in Irgarol 1051 monitoring programs.

(2) Quantification of biofilm mass

The aim of this study was to develop a rapid biochemical test for the quantitative estimation of biofilm mass in the aquatic environment. Biofilms are mainly responsible for the degradation and removal of chemical contaminants from the aquatic environment, and therefore quantification of biofilm mass is critical in the prediction of a chemical fate in the environment. Since biofilms contain relatively large amount of polysaccharide, the determination of total attached polysaccharide can be used as a measure of biofilm mass. Thus, a rapid biochemical method for the quantitative estimation of biofilm mass based on the concentration of polysaccharide was developed. The method was prepared in a detailed step-by-step format, in order to facilitate its technology transfer to industries, universities and government agencies.

(3) Persistence of cyanazine

The persistence of cyanazine in the aquatic environment was studied through experiments on volatility and uptake by bottom-attached biofilms. The results indicate that cyanazine can be considered non-volatile up to 35 °C. Cyanazine was lost from the water in the presence of biofilms. All evidence points towards uptake by the biofilm as the cause of the disappearance of cyanazine. Cyanazine was not biodegraded or biotransformed as indicated by the absence of degradation and/or transformation products. Apparently, cyanazine formed bound residues within the biofilm which could not be extracted, thus increasing its persistence in the aquatic environment.

(4) National survey of Irgarol 1051 in water and sediment

The 1997 national survey of the occurrence of the new antifouling compound Irgarol 1051 in water and sediment from Canadian harbours (Vancouver, Toronto, Windsor, Port Stanley, Midland, Kingston, Montreal, and Halifax) and Japanese harbours (Kobe and Mizushima) has been completed. Analysis of Irgarol in water samples was finished, but that for sediment samples is still on-going due to the tedious extraction procedure. The preliminary results indicated that both the Canadian and Japanese aquatic environments have not been contaminated by Irgarol. Irgarol 1051 has recently been registered in the United States as a replacement chemical for the organotin biocides in antifouling paints, and Canada is seriously considering using it in antifouling formulations. Thus, it is anticipated that the nationwide (Canada and Japan) survey for Irgarol in water and sediment will be continued into the next fiscal year.

Articles/reports/conference presentations in FY 97/98

- (1) Liu, D., R.J. Maguire, Y.L. Lau, G.J. Pacepavicius, H. Okamura and I. Aoyama. 1997. Transformation of the new antifouling compound Irgarol 1051 by *Phanerochaete chrysosporium*. *Water Res.* **31**: 2363-2369.
- (2) Liu, D., R.J. Maguire, Y.L. Lau, G.J. Pacepavicius, H. Okamura and I. Aoyama. 1998. Microbial adsorption of cyanazine and metolachlor. *J. Environ. Sci. Health.* **B33(1)**: 1-15.
- (3) Pacepavicius, G.J., Y.L. Lau, D. Liu, H. Okamura and I. Aoyama. 1997. A rapid biochemical method for estimating biofilm mass. *Environ. Toxicol. Water Qual.* **12**: 97-100.
- (4) Lesage, S., H. Hofmann, W.-C. Li, K. Millar, D. Liu and H. Seidel. 1997. Effects of humic acids on the biodegradation of PAHs by bacteria and fungi. *In* B. Alleman and A. Leeson (eds.), *In Situ and On-Site Bioremediation. Proceedings of the Fourth International Symposium on In-Situ and On Site Bioremediation* **4(2)**: 185-190.
- (5) Lesage, S., W.-C. Li, K. Millar, S. Brown and D. Liu. 1997. Influence of humic acids on the bioremediation of polycyclic aromatic hydrocarbons from contaminated soil and aquifers. pp. 325-338. *In* *Proceedings of the 6th Symposium and Exhibition on Groundwater and Soil Remediation*. Montreal, March 18 - 21.
- (6) Dutka, B.J., D. Liu, A. Jurkovic, R. McInnis, H.-B. Lee, F. Onuska and S.S. Rao. 1997. Observations from a six month study on the effect of biodegradation processes in sediment on toxicity potential of targeted chemicals. *NWRI Contribution No.* 97-208.

- (7) Lau, Y.L., D. Liu and G.J. Pacepavicius. 1998. Persistence of cyanazine in the aquatic environment. NWRI Contribution No. 98-005.
- (8) Liu, D. Toxicity assessment during biodegradation processes. *Presented at the Eighth International Symposium on Toxicity Assessment*, Perth, Australia, May 25-30, 1997.
- (9) Liu, D., R.J. Maguire, Y.L. Lau, G.J. Pacepavicius, H. Okamura and I. Aoyama. Biotransformation of Irgarol 1051. *Presented at the Eighth International Symposium on Toxicity Assessment*, Perth, Australia, May 25-30, 1997.
- (10) Aoyama, I., R. Luo, H. Okamura and D. Liu. Monitoring of toxic chemicals using *Daphnia magna* mobility. *Presented at the Eighth International Symposium on Toxicity Assessment*, Perth, Australia, May 25-30, 1997.
- (11) Okamura, H., I. Aoyama, J. Gaynor, D. Liu and G. Pacepavicius. Effect of natural substances in river water on the photolysis of herbicide. *Presented at the Eighth International Symposium on Toxicity Assessment*, Perth, Australia, May 25-30, 1997.
- (12) Takahashi, N., A. Tanaka, R. Luo, H. Okamura, I. Aoyama and D. Liu. Biodegradation and photolysis of the insecticide 1-(6-chloronicotinyl)-2-nitroiminoimidazolidine (Imidacloprid) and its toxicity. *Presented at the Eighth International Symposium on Toxicity Assessment*, Perth, Australia, May 25-30, 1997.
- (13) Okamura, H., I. Aoyama, R. Luo and D. Liu. Ecotoxicity assessment of landfill leachates at Teshima Island, Japan. *Presented at the International Bioassay Symposium in Toyama*, Toyama, Japan, October 3, 1997.
- (14) Liu, D., G.J. Pacepavicius, R.J. Maguire, Y.L. Lau, H. Okamura and I. Aoyama. Review of cyclone fermentor test for assessing the biodegradability. *Presented at the Thirty-third Central Canadian Symposium on Water Pollution Research*. Burlington, Ontario, February 9-10, 1998.

CURRENT RESEARCH - FY 97/98

PROJECT: Priority Substances - Pathways/Fate
STUDY TITLE: Sources and Fate of Toxic Substances
STUDY LEADER: F.I. Onuska

The study carries out research on fundamental mechanisms governing the persistence and fate of toxic chemicals in aquatic environments. It covers activities focusing on the application of a previously developed demixing technique of two miscible solvents for extracting anilines, benzidines, s-triazine and TNT groups of explosives in water and sediment samples. Major achievements are summarized below:

(1) Explosive materials in the environment

The traditional methodologies for the removal of explosives from soil and sediment matrices prior to their analytical determination are the sonication and the Soxhlet extraction methods. They use large quantities of solvents and are very time-consuming. We demonstrated that the demixing of two soluble solvents is a very effective methodology for the extraction of both classes of explosives from water matrices. For soil and sediment samples, an accelerated solvent extraction method using acetone and methanol (1:1) provides recoveries that are superior to the sonication (U.S. EPA Method 3545) and the Soxhlet methods. The new method is also much more effective as 95-99% recoveries can be obtained in less than 30 minutes vs. 16 to 24 hours for the traditional methods. The effects of various operational parameters such as temperature, pressure and suitability of various solvents were investigated.

As a significant number of polar and thermally unstable pollutants cannot be determined by gas chromatography (HRGC), the use of high performance liquid chromatography (HPLC) was investigated for the detection of highly energetic materials in environmental samples. The first group covers separation of s-triazine explosives such as RDX and HMX and the second group covers separation of nitro derivatives. Detection limits were established for both types and are at 200 ppt levels using UV-absorption detector. Certified samples were used to evaluate precision and accuracy.

(2) Identification of pollutants in an industrial effluent

Some aromatic amines used industrially are highly toxic and carcinogenic. Little is known of their occurrence in the Canadian environment. A method was developed for their extraction and analysis using HRGC-MS. The method was applied to the analysis of an industrial effluent from a pigment-manufacturing company, and to the analysis of influent, effluent and sludge from the sewage treatment plant (STP) receiving the industrial effluent. It was found that the industrial effluent contained relatively high levels of aniline and other aromatic amines as a consequence of the biodegradation of pigments and dyes. Benzidine and 3,3'-dichlorobenzidine are the primary

sources of these pollutants. Benzidine was present in the industrial effluent (5 to 50 ppb). The following pollutants were also identified: aniline (121 ppb); 2,6-dichloroaniline (118 ppb); 2,4,5-trichloroaniline (388 ppb); 1-chloro-3-nitroaniline (120 ppb); and 3,4-dichloroaniline (99 ppb). A 24-hr composite industrial sample showed a significant quantity of benzidine (0.8 ppb); 3,3'-dichlorobenzidine (106 ppb) and 3,3'-dimethoxybenzidine (2.9 ppb). Samples of STP influent and effluent revealed between 0 to 2 ppb benzidine; the 2,4-dinitroaniline concentration in the influent was up to 1960 ppb. This work is being done for Ontario Region, which is responsible for the strategic options process for 3,3'-dichlorobenzidine as a result of the CEPA PSL1 assessment.

(3) Other work

An attempt was made to analyze alkyltin pollutants by ITP-CZE and micellar electrokinetic chromatography using direct and indirect UV-detection. Tributyltin and triphenyltin were studied as model compounds and a 3 mM solution of 4-aminopyridine was used for indirect detection at 261 nm. This technique was found to be unsuitable for environmental monitoring due to its lack of sensitivity.

We also evaluated the use of HRGC-MS for quantitation of derivatized organotin compounds, especially butyltin compounds which we derivatized using sodium tetraethylborate. This reaction is very quick and the limit of detection is around 200 ppt. In this case, selected ion monitoring must be utilized and a complete speciation of inorganic and organotin compounds can easily be achieved.

Simulation software for advanced protocol methodology development is fully functional and is being utilized for the prediction and simulation of ITP and CZE separations. The software uses steady-state algorithms.

Articles/reports/conference presentations in FY 97/98

- (1) Onuska, F.I. and R.J. Maguire. 1997. Environmental application of mass spectrometry: toxaphene analysis. pp. 533-558. *In* R.M. Caprioli (ed.) *Selected-Topics and Mass Spectrometry in the Biomolecular Sciences*. NATO-ASI Series in Mass Spectrometry. Kluwer Academic Publishers, Amsterdam, The Netherlands.
- (2) Krupcik, J., E. Benicka, J. Hrouzek and F.I. Onuska. 1997. Determination of electron capture detector relative response factors for HRGC quantitative trace analysis of polychlorinated biphenyls. *Petroleum and Coal* **39**(2): 48-51.
- (3) Dutka, B.J., D. Liu, R. McInnes, A. Jurkovic, R. McInnes, F.I. Onuska and S.S. Rao. 1997. Observations from a six months study on the effect of biodegradation process in sediment on toxicity potential of targeted chemicals. NWRI Contribution No. 97-208.
- (4) Onuska, F. I., K.A. Terry and R.J. Maguire. 1997. High resolution gas chromatography-mass spectrometry of some benzidines in industrial waste water. NWRI-Contribution No 97-202.

- (5) Onuska, F.I., D. Kaniansky, K.D. Onuska and M.L. Lee. Isotachophoresis: Trials, tribulations and trends in trace analysis of organic and inorganic pollutants. *Presented at the 19th International Symposium on Capillary Chromatography and Electrophoresis*, Wintergreen, VA, May 1997.
- (6) Onuska, F.I. Isotachophoresis: Applications in environmental analysis. *Presented at the Latin American Congress in Chromatography (COLACRO 7)*, Aquas de São Pedro, Brazil, March 20-27, 1998.
- (7) Vieira, E.M. and F.I. Onuska. Determination of 1,3,5-trinitro-1,3,5-triazacyclohexane (rdx), 1,3,5,7-tetranitro-1,3,5,7-tetraazacyclooctane (hmx) and 2,4,6-trinitrotoluene in water. *Presented at the Latin American Congress in Chromatography (COLACRO 7)*, Aquas de São Pedro, Brazil, March 20-27, 1998.
- (8) Onuska, F.I. Basics in isotachophoresis: practical guide for separation of anions and cations. 62 pp. Guide distributed to participants at a short course on electrophoresis (in Portuguese). Latin American Congress in Chromatography (COLACRO 7), Aquas de São Pedro, Brazil, March 20-27, 1998.

CURRENT RESEARCH, FY 97/98

NON-POINT SOURCES OF POLLUTION AQUATIC ECOSYSTEM PROTECTION BRANCH

The project goal is to develop knowledge for Environment Canada to manage sustainable large-scale ecosystem impacted by stresses from non-point sources of pollution from agriculture, industry and urban developments. Knowledge generated from this research is applied in the development of water management plans for large ecosystems, such as those currently studied under the Fraser River Action Plan (FRAP) and the Great Lakes 2000 Program.

CURRENT RESEARCH, FY 97/98

PROJECT: Non-Point Sources of Pollution
STUDY TITLE: Prevention and Remediation of Urban Non-Point Sources of Pollution
STUDY LEADER: J. Marsalek

Research was conducted on the sources and impacts of urban non-point sources of pollution and the means of prevention or remediation of such impacts.

(1) Stormwater and combined sewer overflow (CSO) pollution and impacts on receiving waters

Several studies were undertaken to address these important concerns. Heavy metals and polycyclic aromatic hydrocarbons (PAHs) in stormwater runoff from a highway bridge were studied in Burlington. The highest mean concentrations in whole water samples were found for Zn, Cu and Pb (0.337, 0.136 and 0.072 mg/L), and up to 45% of these burdens were in the dissolved phase. Mean PAH event-mean concentrations in whole water samples ranged from 0.015 to 0.5 µg/L, with the dissolved phase representing less than 10% of these burdens. Highway sediment was grossly polluted with respect to Zn, Cu and Pb. The highway runoff chemistry indicated that uncontrolled discharges of highway runoff impact on receiving water quality and may require remediation by stormwater management practices.

General aspects of wet-weather pollution impacts were addressed in a review paper, and more specific aspects were addressed in a Chapter for the NATO Advanced Study Institute (ASI) proceedings on Hydroinformatics and through a NATO Linkage Grant with the Slovak Technical University in Bratislava. The finding reported indicate the need for an integrated approach to impact assessment, considering all pollution sources. Among the various sources of urban stormwater, highway runoff was found very important in terms of toxic impacts. With respect to stormwater and CSO impacts, both temporal and spatial characteristics are of great importance. Temporal characteristics are reflected in acute or cumulative impacts. Acute impacts include acute toxicity, flooding, dissolved oxygen depletion, and fecal bacterial pollution. Cumulative impacts arise from a gradual build up of pollutants in the receiving water and become apparent only after such accumulations exceed some critical threshold values. Thus, with respect to acute impacts, flow, flux and pollutant variations in time are important, as well as variations in ambient conditions in the receiving waters. For the cumulative impacts, time-integrated data are adequate.

Toxicity of municipal effluents, including stormwater, CSO and UV disinfected effluents, has been assessed at more than 15 sites in two important Areas of Concern, Hamilton Harbour and Toronto Waterfront. Using a battery of bioassays, various sources of municipal effluents were assessed. This assessment indicates that in general, toxicity was found more frequently in stormwater rather than in CSOs, and it varied in time and space. Temporal variations of stormwater toxicity corresponded to typical pollutant fluxes, characterized by the first flush and high concentrations of chemicals during the early stages of runoff. While several bioassays indicated toxicity of the first flush, most of runoff outside of the first flush was non-toxic. With respect to space, the controlling factor was the source of

runoff. The highest toxicity was found in highway runoff, particularly in the case of highways with high volumes of traffic. It appears that the first flush of runoff from a freeway ($> 100,000$ vehicles/day) is toxic, or even severely toxic. The stormwater toxicity was somewhat reduced by Best Management Practices (BMPs), such as ponds, biofilters and constructed wetlands, but the results were not consistent and further research is needed. CSOs were generally less toxic than stormwater. Variations among the CSO sites sampled were observed, depending on local sources of stormwater and municipal sewage. Higher incidence of toxic samples was observed at two overflows with industrial or hospital/university inputs of wastewater. Effects of treated effluent disinfection by UV were studied at three sewage treatment plants (STPs) of various sizes. For the conditions studied, no increase in effluent toxicity was observed, as a result of UV disinfection. In that sense, the UV results were comparable to those obtained by careful effluent chlorination and dechlorination; chlorine overdose or incomplete dechlorination may result in residual toxicity.

(2) Prevention or remediation of stormwater and CSO pollution

Stormwater quality can be enhanced by such BMPs as ponds and oil/grit separators (OGS). The sustainability of such measures needs to be addressed and has to deal with an environmentally safe disposal of contaminants removed from stormwater at these facilities. CSO pollution is generally controlled by treatment, and among the various treatment processes proposed, chemically aided hydrodynamic separation can achieve good removals of suspended solids. In areas with recreational beaches, such treatment should also include environmentally safe disinfection by UV irradiation.

Through a cost-recovery arrangement with the Ontario Concrete Pipe Association (OCPA), the OCPA OGS was tested and found deficient in two aspects, i.e., poor performance of the treatment chamber arising from an unfavourable flow distribution, and the risk of trapped oil re-entry into the upstream sewer. Both problems were solved by redesigning the separator chamber inlet - removing the vertical inlet elbow causing flow eddying and replacing it with a straight inflow pipe fitted with an energy dissipator in the form of a semicircular baffle, and a back-flow check valve. While for low flows (<10 L/s), the new and old designs performed similarly and were both effective in coarse sediment and free oil removal, for high flows (up to 50 L/s), only the new design achieved good pollutant removal and outperformed the original design by a large margin.

A major paper was published on pollutant removal by stormwater ponds and outlined the methodology for assessing BMP performance. Towards this end, it is important to define various pond regimes, with respect to loading and in-pond processes, particularly those occurring during wet-weather events and during baseflow. With more than 500 samples collected and analyzed, it was possible to establish the pond's mass balances with respect to flow and fluxes of various materials and pollutants (suspended solids, heavy metals, nutrients, some organics, and bacteria). Dissolved constituents exhibited zero removals during baseflow, but some positive removal during storm events; nutrients and suspended solids exhibited negative removal during baseflow (incoming polluted water was displacing cleaner water in the pond) and positive removal during events, and metals and organics displayed positive removals during both baseflow and storm events. Constituent removal was controlled mostly by physical processes (sedimentation). Additional information was obtained on distributions of water quality parameters. The data originating in systems with large storage and intense mixing (e.g. pond outflow) were normally distributed; other (e.g. pond inflow) were lognormally distributed. This finding has implications for future studies of stormwater quality, and particularly their experimental design.

Recognizing that settling is the most important pond process, it can be expected that well functioning ponds accumulate large quantities of polluted sediment. The properties of such sediment were studied at several facilities and described in terms of their physical and chemical characteristics and toxicology. It appears that urban ponds accumulate mostly fine materials (silt and clay), these materials are marginally polluted, and may require special considerations for disposal. Pond sediments should be periodically removed to sustain pond performance in pollutant removal. Two criteria are used for deciding on sediment removal - loss of 10% of active pond volume, or a loss of sediment removal performance (by 5%). An additional criterion was proposed, the level of contamination of the pond sediment. Relatively infrequent sediment removals (once in 15-30) years are expected.

Articles/reports/conference presentations in FY 97/98

- (1) Averill, D., D. Mack-Mumford, J. Marsalek, R. Andoh and D. Weatherbe. 1997. Field facility for research and demonstration of CSO treatment technologies. *Water Sci. Tech.* **36(8-9)**: 391- 396.
- (2) Anderson, B.C., D.W. Kantor, W.E. Watt and J. Marsalek. Bioleaching of trace metals from stormwater detention pond sediments. *Presented at the CSCE/ASCE Environmental Engineering Conference*, Edmonton, Alberta, July 10-15, 1997.
- (3) Chambers, P.A., M. Allard, S.L. Walker, J. Marsalek, J. Lawrence, M. Servos, J. Busnarda, K.S. Munger, K. Adare, C. Jefferson, R.A. Kent and M.P. Wong. 1997. Impacts of municipal wastewater effluents on Canadian waters: a review. *Water Qual. Res. J. Canada* **32(4)**: 659-713.
- (4) Larkin, G.A. and J. Marsalek. 1997. Laboratory testing of the Ontario Concrete Pipe Association Oil/Grit Separator. AEPB Technical Report No. TN97-005.
- (5) Larkin, G.A. and J. Marsalek. 1998. Laboratory Testing of Modifications to the OCPA Oil/Grit Separator (Phase II). AEPB Technical Report No. TN98-001.
- (6) Marsalek, J. 1998. Challenges in Urban Drainage: Environmental Impacts, Impact Mitigation, Methods of Analysis and Institutional Issues. Chapter 1, NATO ASI on Hydroinformatics Tools for Planning, Design, Operation and Rehabilitation of Sewer Systems. NWRI Contribution No 98-010.
- (7) Marsalek, J., B. Brownlee, T. Mayer, S. Lawal and G.A. Larkin. 1997. Heavy Metals and PAHs in Stormwater Runoff from the Skyway Bridge, Burlington, Ontario. *Water Qual. Res. J. Canada* **32(4)**: 815-827.
- (8) Marsalek, J. and D. Doede 1997. Hydraulic assessment of the WTI Plate clarifier: flow capacity and outflow zone velocity field. AEPB Technical Report No. TN97-002.
- (9) Marsalek, J., B.G. Krishnappan, W.E. Watt and B.C. Anderson. 1998. Size Distribution of Suspended Sediments in an On-Stream Stormwater Management Pond. NWRI Contribution No. 98-009.
- (10) Marsalek, J. and G.A. Larkin. 1997. Stormwater Quality Management: The Role of Oil/Grit Separators and Laboratory Investigations of the OCPA Off-Line Oil/Grit Separator. NWRI Contribution No. 97-214.

- (11) Marsalek, J. and G.A. Larkin. 1998. Sustainability of Stormwater Ponds: Addressing the Sediment Issues. NWRI Contribution No. 98-017. (Distributed at the SWAMP conference, Toronto, Ontario, Feb. 23 - 24, 1998.)
- (12) Marsalek, J., C. Maksimovic, E. Zeman and R. Price. 1998. Hydroinformatics Tools for Planning, Design, Operation and Rehabilitation of Sewer Systems. ISBN- 0-7923-5097-9. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- (13) Marsalek, J. and W. Schilling. 1998. Operation of sewer systems. Chapter 6, NATO ASI on Hydroinformatics Tools for Planning, Design, Operation and Rehabilitation of Sewer Systems. NWRI Contribution No 98-013.
- (14) Mayer, T., B. Dutka and J. Marsalek. 1997. Toxicity and contaminant status of suspended and bottom sediments from urban stormwater ponds. pp.159-165. *In* J.S. Goudey, S.M. Swanson, M.D. Treissman and A.J. Niimi (eds.), Proc. 23rd Annual Aquatic Toxicology Workshop, Calgary, Alberta.
- (15) Rochfort, Q., J. Marsalek, J. Shaw, B. Dutka, B. Brownlee, A. Jurkovic, R. McInnis and G. MacInnis. 1997. Acute Toxicity of Combined Sewer Overflows and Stormwater Discharges. NWRI Contribution No. 97-190.
- (16) Rochfort, Q., I. Scott, R. McInnis, A. Jurkovic, J. Marsalek and M. Servos. 1998. Chronic toxicity of high and low intensity UV disinfected effluents from three sewage treatment plants in Ontario: data report. NWRI Contribution No. 98-015.
- (17) Sztruhar, D., M. Sokac, J. Marsalek, E. Frankova, L. Hyanek, D. Rusnak, S. Stanko, J. Ilavsky and J. Namer. 1997. A case study of combined sewer overflow pollution: assessment of sources and receiving water effects. *Water Qual. Res. J. Canada* **32(3)**: 563-578.
- (18) Sztruhar, D., M. Sokac, L. Hyanek, N. Frankova, D. Rusnak, J. Namer, S. Stanko and J. Marsalek. 1997. Conjunctive monitoring of a sewer system and receiving waters in a medium size community. *Water Sci. Tech.* **36(8-9)**: 271- 276.
- (19) Van Buren, M.A., W.E. Watt and J. Marsalek. 1997. Removal of selected urban stormwater constituents by an on-stream pond. *Journal of Environmental Planning and Management* **40**: 5-18.
- (20) Van Buren, M.A., W.E. Watt and J. Marsalek. 1997. Application of the log-normal and normal distributions to stormwater quality parameters. *Water Research* **31**: 95-104.

CURRENT RESEARCH, FY 97/98

PROJECT: Non-Point Sources of Pollution
STUDY TITLE: Aquatic Sediment Structure: Implications for Non-Point Source Sediment and Contaminant Transport.
STUDY LEADER: I. G. Droppo

Research was completed and/or is ongoing in the area of floc architecture and its influence of floc function/behaviour, stormwater management study of coal pile runoff, transport/erosion characteristics of coal deposits, and combined sewer overflows and stormwater runoff studies for the Kenilworth industrial boat slip. Major activities are summarized below:

(1) Floc structure/behaviour

Flocculated fine-grained sediment constitutes a very complex matrix of microbial communities and organic (detritus, cellular debris and extracellular polymers) and inorganic material. 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 US-EPA Priority Pollutants and many other unlisted but environmentally sensitive chemicals, have environmental pathways that are primarily or exclusively associated with inorganic and bioorganic particles/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. This study investigates the complex structural matrix of riverine flocs over a large range of magnifications using correlative microscopic techniques. A better understanding of the structural components of flocculated suspended sediment and their functional relationships with one another will help improve our knowledge of floc behaviour in natural and engineered systems.

(2) Stormwater management of coal pile runoff

Hamilton Harbour, Ontario, is one of 43 Areas of Concern (AOC) around the Great Lakes designated by the International Joint Commission (IJC) due to environmental impairments. Contributing to these impairments are point source inputs that discharge suspended solids, trace metals and organic compounds from several industrial sites into the Harbour. One source of suspended solids, trace metals and organic compounds is runoff from coal piles, which results when coal stockpiled for the steel industry is exposed to rainfall. The Kenilworth boatslip has been identified as a 'hotspot' within Hamilton Harbour because of highly contaminated bottom sediments. The objective of this study was to determine suspended solids, trace metals and polynuclear aromatic hydrocarbons (PAHs) concentrations and loadings in the coal pile runoff that discharges to the Kenilworth boatslip. Flow-proportioned samples of the runoff were collected from April through October 1996 and analyzed for total suspended solids, selected trace metals and PAHs. The suspended solids and total trace metal levels (Al, Fe, Mn, V, Cd, Cr, Cu,

Pb, Zn) observed in coal pile runoff often exceeded Canadian Water Quality Guideline levels. Levels for four of five PAHs (fluoranthene, phenanthrene, pyrene, chrysene) exceeded the provincial 'severe effect level' (SEL) for PAHs in sediment. Given the high levels and loadings of solids, metals, and PAHs and the relatively low volume of runoff, it has been suggested that the coal pile discharge be treated as a remedial action for the Harbour.

(3) Transport characteristics of coal deposits within Hamilton Harbour

The runoff from an industrial coal pile has contributed to highly contaminated bed sediment in the Kenilworth boatslip with significant concentrations of coal tar. Because there is busy ship traffic within the boatslip, concern has been raised over the possibility of the resuspension of this contaminated sediment by propeller wash, with its eventual transport to, and contamination of, cleaner areas of the Harbour.

Experiments on the erosion characteristics of contaminated bed sediments from Hamilton Harbour were conducted in an annular flume. Sediments were obtained from the Kenilworth boatslip that receives coal pile runoff. Values of the critical shear stress for bed erosion were obtained for nine different runs. 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. Size distributions for the sediment flocs were obtained and discussed based on a conceptual model of floc recycling. The results of this study are relevant to any aquatic environment where sediment remobilization is of concern and should be considered when the transport of sediments and associated contaminants are being modelled.

(4) Combined sewer overflows and stormwater runoff

This study stems from the need to assess the current inputs and fate of contaminants to an Area of Concern from a variety of sources in order to ensure that any proposed remediation will produce the desired results. This study focused specifically on an industrial boatslip of Hamilton Harbour. The major sources of contaminants include: combined sewer overflows (CSO) from the Kenilworth Sewershed, coal pile runoff and direct industrial cooling water discharges (monitored by the MISA program of the MOE). 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 the Kenilworth boat slip. Quantitative source evaluations using the methods described in this study are strongly recommended prior to the planning and implementation of habitat and sediment remediation work.

Articles/reports/conference presentations in FY 97/98

- (1) Droppo, I.G., G.G. Leppard, D.T. Flannigan and S.N. Liss, 1997. The freshwater floc: A functional relationship of water and organic and inorganic floc constituents affecting suspended sediment properties. *Water Air Soil Poll.* **99**: 43-53.

- (2) Irvine, K.N., I.G. Droppo, T.P. Murphy and A. Lawson. 1997. Sediment resuspension and dissolved oxygen levels associated with ship traffic: Implications for habitat remediation. *Water Qual. Res. J. Canada* **32(2)**: 421-437.
- (3) Droppo, I.G., D. Jeffries, C. Jaskot and S. Backus. Freshwater flocculation in cold regions: A case study from the Mackenzie River Delta, Northwest Territories, Canada. Arctic. (*in press*)
- (4) Bura, R., M. Cheung, B. Liao, J. Finlayson, B.C. Lee, I.G. Droppo, G.G. Leppard and S.N. Liss. Composition of extracellular polymeric substances in the activated sludge floc matrix. *Water Sci. Technol.* (*in press*).
- (5) Finlayson, J.C., B. Liao, I.G. Droppo, G.G. Leppard and S.N. Liss. The relationship between the structure of activated sludge flocs and the sorption of hydrophobic pollutants. *Water Sci. Technol.* (*in press*).
- (6) Leppard, G.G., I.G. Droppo and S.N. Liss. Microbial floc structure, adhesion and biofilm formation. *Presented at the International Conference on Biofilms in Aquatic Systems*, University of Warwick, Warwick, U.K., April 13-16, 1997.
- (7) Droppo, I.G. Implications of using effective vs. absolute particle size distributions for sediment transport studies. *Presented at the Middle States Division - Association of American Geographers and Canadian Association of Geographers - Ontario Division (CAGONT) Joint Meeting*, State University College at Buffalo, Buffalo, NY, USA, October 17-18, 1997.
- (8) Droppo, I.G., D.E. Walling and E.D. Ongley. 1997. Suspended Sediment Structure: Implications for sediment and contaminant transport modelling. NWRI Contribution No. 97-209.
- (9) Droppo, I.G., K.N. Irvine, T.P. Murphy and C. Jaskot. 1997. Fractionated metals in street dust of a mixed land use sewershed, Hamilton, Ontario, Canada: Spatial patterns and implications for CSO impacts on receiving water body quality. NWRI Contribution No. 97-210.
- (10) Petticrew, E.L. and I.G. Droppo. 1998. Sediment flocculation: An important consideration for transport studies in fluvial geomorphology. NWRI Contribution No. 98-007.
- (11) Curran, K.J., I.G. Droppo, K.N. Irvine and T.P. Murphy. 1998. Suspended solids, trace metals, and PAH levels and loadings from coal pile runoff to Hamilton Harbour, Ontario. NWRI Contribution No. 98-002.
- (12) Lau, Y.L. and I.G. Droppo. 1998. Flume experiments with Hamilton Harbour coal pile deposits. NWRI Contribution No. 98-024.

CURRENT RESEARCH, FY 97/98

PROJECT: Non-Point Sources of Pollution
STUDY TITLE: Statistical Modelling of Environmental Changes
STUDY LEADER: A.H. El-Shaarawi

Research activities were focused on reviewing and developing methods for modelling the spatial and temporal changes in water quality of the Great Lakes. Selected case studies were used to demonstrate the power of the developed methods in advancing our understanding of the significance of water quality changes and their causes. In addition, the applications were also used as a framework for technology transfer to the users. An overview is presented below.

(1) Evaluation of space-time modelling methods

The performance of existing methods for space-time modelling has been evaluated using data from two Lake Ontario monitoring programs: the Bio-Index Program and the Water Quality Monitoring Program. The Bio-Index Program does not provide a good spatial coverage of the lake since its data are collected from only two sampling stations (station 41 and 81), but it provides good time series data. This is in contrast with the regular Water Quality Monitoring Program where the emphasis is on spatial coverage; more stations are sampled but less frequently. The evaluation revealed that the currently available methods were not adequate for modelling the two types of data due to their inability to handle irregularly spaced time series data, missing and below detection values, and to model spatial auto-correlation especially for count data. New methodologies which are tailored to the types of data generated by our monitoring programs have been developed and applied to:

- Measurements made on 10 water quality variables from March 16, 1981 to October 1995 at stations 41 and 81 in Lake Ontario: The results show a major decrease in the concentration of these variables.
- Three size classes phytoplankton biomass measurements at 40 sampling stations in Lake Ontario in 1990: The model indicates strong spatial dependence among the sampling stations.

These models will be used to study the efficiency of the two programs in their ability to describe the current status, and to detect and estimate trends in water quality.

(2) Niagara River trends

A comprehensive review of the statistical modelling methodologies developed for assessing contaminant trends in the Niagara River was presented to delegates from Environment Canada, the Ontario Ministry of the Environment, the US-EPA and the New York Department of Environmental Conservation attending a workshop at the Canada Centre for Inland Waters, December 1-2, 1997. The four agencies are responsible for reporting progress in achieving the goals of the 1987 Canada - U.S. Niagara River Declaration of Intent. The first day was focused

on the modelling approach, on assessing assumptions and on interpreting the results of the analysis, followed by a software demonstration. The second day was devoted to answering questions; participants also recommended the production of a user friendly manual for the software. These methodologies will be used by the four Parties to prepare their yearly report on contaminant trends in the Niagara River.

Articles/reports/conference presentations in FY 97/98

- (1) El-Shaarawi, A.H. and R. Viveros. 1997. Inference about the mean in log-regression with environmental applications. *Environmetrics* 8(6): 569- 582.
- (2) El-Shaarawi, A.H. 1998. *Environmetrics*. pp. 202-203. In S. Kotz, C.B. Read and D.L. Banks (eds.) *Encyclopedia of Statistical Sciences, Update Volume 2*. John Wiley & Sons, New York.
- (3) Muller, I. and A.H. El-Shaarawi. 1998. Various confidence intervals for the linear calibration estimator. *J. Stat. Plan & Inf.* (in press)
- (4) Barton, D., D.R. Brillinger, A.H. El-Shaarawi *et al.* Final Report of the Haystack Orbital Debris Data Review Panel. NASA JSC Report (in press)
- (5) El-Shaarawi, A. H., S.R. Esterby and M.B. Abdullah (eds.). 1998. Statistical methods for environmental sciences. *Environmetrics* Volume 9 Number 1.
- (6) Williams D. J., K. Kuntz, L. Richman, G. Hitchin and A. H. El-Shaarawi. Have Remedial Actions Worked in the Niagara River? *Presented at the* Public Meeting on the Niagara River, Niagara Falls NY, March 10, 1998.
- (7) El-Shaarawi, A. H. Space-Time Modelling of Environmental Changes. *Presented to* Iowa State University, April 7, 1997.
- (8) El-Shaarawi , A. H. Space-Time Modelling of Water Quality Changes in Large Lakes. *Presented at* IAGLR 97, Buffalo, NY, June 1-5, 1997.
- (9) Kuntz, K. , A.H. El-Shaarawi and D. Williams, Trends in Contaminants in the Niagara River since 1986. *Presented at* IAGLR 97, Buffalo, NY, June 1-5, 1997.
- (10) L'Italian, C.S. and A.H. El-Shaarawi. Multivariate Analysis in the Presence of Missing Data with Applications to Lake Ontario Monitoring Data. *Presented at* IAGLR 97, Buffalo, NY, June 1-5, 1997.
- (11) El-Shaarawi, A. H. Log-Linear Model for Correlated Count Data. *Presented at the* International Conference on Combinatorics, Information Theory and Statistics, University of Southern Maine, USA, July 18-20, 1997.
- (12) Zapotosky J.E., A. H. El-Shaarawi, R. G. Drexler and I. Muller. Temporal Variability of Simulated Body Currents along Horizontal Grounding Terminals. *Presented at the* Environmetrics Conference, Innsbruck, Austria. August 4 - 8, 1997.
- (13) El-Shaarawi, A. H., and Muller, I. Application of Bootstrap to the Calibration Problem with Environmental Examples. *Presented at the* Environmetrics Conference, Innsbruck, Austria. August 4 - 8, 1997.

- (14) Marsalek, J., S. Lawal, A.H. El-Shaarawi and B.J. Dutka. Guidelines for Recreational Microbiological Water Quality: Challenges in Water Management Applications. *Presented at the Environmetrics Conference, Innsbruck, Austria. August 4 - 8, 1997.*
- (15) El-Shaarawi A.H. Discussant of the invited three papers session on Environmental Sampling, Monitoring and Assessment *Presented at 51st Session of the International Statistical Institute, Istanbul, Turkey, August 18-26, 1997.*
- (16) El-Shaarawi, A.H. Statistical Considerations in Environmental Impact Assessment. *Presented at the Water and Statistics Conference, Ankara, Turkey, August 28-30, 1997.*
- (17) El-Shaarawi, A.H. Statistical Issues in Water Quality Modelling. *Presented at the Conference on Recent Advances in Statistics and Probability, Calcutta, India, December 29, 1997 - January 1, 1998.*
- (18) El-Shaarawi, A.H. On Statistical Calibration. *Presented at McMaster University, Hamilton, Ontario, February 3, 1998.*

CURRENT RESEARCH, FY 97/98

PROJECT: Non-Point Sources of Pollution
STUDY TITLE: Transport Characteristics of Fraser River Sediment and the Sediment/Contaminant/Biota Interactions.
STUDY LEADER: B.G. Krishnappan

Fine sediments play a major role in the transportation of hydrophobic contaminants in river systems and hence the knowledge of their transport characteristics is an essential prerequisite for modelling contaminant transport and interactions with biota. In this study, the transport characteristics of the Fraser River sediment were studied both in the laboratory and in the field. In addition, transport characteristics of fine sediment from a variety of environmental settings including a tile drain in an agricultural watershed and a stormwater pond in Kingston, Ontario were systematically studied in the laboratory. Some of the significant results of the current studies are outlined below:

(1) Transport characteristics of fine sediments in the Fraser River system

Transport characteristics of fine sediment from the Fraser and Thompson rivers were studied as part of the FRASER RIVER ACTION PLAN. The studies consisted of field surveys and laboratory investigations. In the field surveys, the in-situ size distributions of the suspended sediments of the Fraser and Thompson rivers were measured using a laser device and were compared with the size distribution of the dispersed primary particles. From such comparisons, the flocculation state of the sediments was assessed. The laboratory investigations involved measurement of deposition and erosion rates of the river sediment under controlled conditions in the rotating circular flume. In addition, the influence of the pulp mill effluent on the flocculation mechanism of the sediment was also investigated in the laboratory. Based on the results of these investigations, a new mathematical model of the fine sediment transport was formulated for the Fraser river system.

(2) A laboratory investigation of depositional characteristics of mud from an inland harbour using a rotating circular flume

Many hydrophobic contaminants are attached to fine sediments which are transported into Great Lakes harbours by the tributaries. Settling of sediment in the harbours depends on physical, chemical and biological factors. An important aspect of modelling water quality and the transport of the contaminants is the formation of flocs and the resultant effects on settling velocity and deposition rates. These processes cannot be estimated theoretically. For this study, we used our unique rotating flume to study the effects of turbulence on floc formation and settling velocity for sediment from Port Stanley harbour. This study adds to our knowledge of the effects on floc formation of shear stress, seasonality, and storage time as they relate to the transport properties of fine sediment from Port Stanley harbour. Sample storage in the flume resulted in increased

flocculation for the two finer samples. A sample with a higher percentage of silt did not flocculate to the same extent as the finer samples. Flocculation was also influenced by the presence of bacteria; however further investigation is required to determine their relative importance compared to the presence of fines.

(3) Transport characteristics of tile-drain sediments in clay loam soils

Increasing use of tile drains for subsurface drainage in agricultural watersheds has created concern for the delivery of sediment to receiving waters and potential undesirable effects on surface and subsurface water quality. In this study, transport characteristics of sediment from tile drains in predominantly clay-loam soils of a southern Ontario watershed were tested in a rotating circular flume. Tile-drain sediments were collected and mixed with river water at different speeds in the flume to study transport processes such as deposition, erosion and flocculation as a function of bed shear stress. Empirical relationships were developed to describe erosion and deposition processes of tile drain sediment. The relationships are in a form suitable for use in the fine sediment transport model developed by Krishnappan et al (1997) and can be used to predict transport characteristics of tile drain sediment in receiving streams.

(4) Kingston stormwater pond study

This collaborative study with Dr. Jiri Marsalek and Dr. Y.L. Lau (NWRI) was aimed at characterising the size and density distribution of suspended sediment flocs in a stormwater settling pond in Kingston, Ontario. The size distributions of the sediment in suspension were measured using a submersible laser particle size analyser and a relationship between the floc size and the density of the flocs was established on the basis of experiments carried out in the rotating flume. These parameters are useful for establishing the deposition rate of the sediment entering the pond.

Articles/reports/conference presentations in FY 97/98

- (1) Krishnappan, B.G., R.Stephens, B.Moore and J.Kraft. 1997. Transport Characteristics of Fine Sediments in the Fraser River System. NWRI Contribution No. 97-220.
- (2) Skafel, M.G. and B.G. Krishnappan. A laboratory Investigation of Depositional Characteristics of Mud from an Inland Harbour using a Rotating Circular Flume. Water Air Soil Poll. (*in press*)
- (3) Lau, Y.L. and B.G. Krishnappan. 1997. Measurement of Size Distribution of Settling Flocs. NWRI Contribution No. 97-223.
- (4) Marsalek, J., B.G. Krishnappan, W.E. Watt and B.C. Anderson. Size Distribution of Suspended Sediments in an On-Stream Stormwater Management Pond. NWRI Contribution No. 98-009.

- (5) Gharabaghi, B., W.T. Dickinson, R.P. Rudra, W.J. Snodgrass and B.G. Krishnappan. 1998. Effectiveness of Rolled Erosion Control Products in Pre-vegetated Conditions. pp 13-28. *In* Proceedings, Conference 29, International Erosion Control Association, Reno, Nevada, U.S.A.

CURRENT RESEARCH, FY 97/98

PROJECT: Non-Point Sources of Pollution
STUDY TITLE: Sediment/ Contaminant Transport in Rivers
STUDY LEADER: Y.L. Lau

Research was conducted into the effect of biofilm growth on the erodibility of bottom sediments and the persistence of cyanazine and Irgarol in the aquatic environment. Experiments were carried out to investigate the erosion characteristics of contaminated bed sediments from Hamilton Harbour. A study into the transport of sediments in streams with steep slopes was initiated.

(1) Erosion characteristics of coal pile deposits

The Kenilworth boatslip in Hamilton Harbour receives direct industrial discharges (primarily from steel manufacturing), combined sewer overflows and stormwater runoff, and has been designated as one of the "hot spots" of the Hamilton Harbour Remedial Action Plan (RAP). One of the most significant inputs of these contaminants is from direct coal pile runoff which results when coal stockpiled along the side of the boatslip is exposed to rainfall. This runoff creates a highly contaminated sediment bed on the bottom of the boatslip with significant concentrations of coal tar. Because there is busy ship traffic within the boatslip, concern has been raised over the possibility of the resuspension of this contaminated sediment by propeller wash, with its eventual transport to and contamination of cleaner areas of the Harbour.

Experiments on the erosion characteristics of contaminated bed sediments from Hamilton Harbour were conducted in an annular flume. Sediments were obtained from the Kenilworth boatslip which receives coal pile runoff. Values of the critical shear stress for bed erosion were obtained for nine different runs. 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. Size distributions for the sediment flocs were obtained and discussed based on a conceptual model of floc recycling. The results of this study are relevant to any aquatic environment where sediment remobilization is of concern and should be considered when the transport of sediments and associated contaminants are being modelled.

(2) Effect of biofilm growth on critical shear stress

The shear stress at which sediments on a river bed begins to be eroded is called the critical shear stress. As the fate of many toxic contaminants is directly related to the fate of aquatic sediments, the critical shear stress is an important parameter for the investigation of contaminant as well as sediment pathways. Published values for the critical shear stress have all been obtained in the laboratory using clean sand. There is qualitative evidence that the extracellular polymer substances produced by bacteria in biofilms can bind sediment particles and thus increase the shear stress required for initiation of sediment motion. However, quantitative information is lacking and

experiments were designed to obtain such information. Experiments were conducted with bacterial biofilms developed on a sand bed indoors as well as natural biofilms developed outdoors. Further experiments are planned.

(3) Persistence of cyanazine and Irgarol in the aquatic environment

The herbicide cyanazine is a selective triazine herbicide used for annual broadleaf and grass weed control in corn, sorghum, potatoes, soybeans and triazine-resistant canola. It is one of the most heavily used agricultural pesticides in Ontario and yet there is virtually no information on its aquatic fate. Both the U.S. Environmental Protection agency and the Ecosystem Interpretation Branch of Environment Canada have noted that information is required on the occurrence, persistence, fate and toxicity of cyanazine. The persistence of cyanazine was studied through experiments on volatility and uptake by bottom-attached biofilms. The results indicate that cyanazine can be considered non-volatile up to 35 °C. Cyanazine was lost from the water in the presence of algal or bacterial biofilms. All evidence points towards uptake by the biofilm as the cause of the disappearance of cyanazine. However, no degradation products were found showing that the cyanazine could not be biodegraded or biotransformed. Cyanazine formed bound residues within the biofilm which could not be extracted, thus increasing its persistence in the aquatic environment. The results of this work will be useful for determining the hazards posed by cyanazine to the aquatic environment.

Irgarol is a newly developed herbicidal additive for use in copper-based antifouling paints. It is intended for replacing the agent tributyltin which has been banned internationally. However there is no information in the open literature on the persistence and degradation of Irgarol and this hinders the assessment of its ultimate impact on the environment. A study was conducted which showed that mercuric chloride was capable of rapidly catalyzing the hydrolysis of Irgarol 1051 in distilled water and buffer solutions. A mechanism for the HgCl_2 -catalyzed hydrolysis of Irgarol 1051 was proposed. One practical aspect of this work is that HgCl_2 should not be used in preserving water samples in Irgarol 1051 monitoring programs. Further investigations on its persistence is continuing.

(4) Effect of channel slope on the critical shear stress

Effective aquatic ecosystem protection requires better understanding of the sediment transport processes at the bed of steep streams. Movement of gravel and the intrusion of sediment fines into the gravel bed greatly restrict transport of dissolved oxygen into and through the bed substrate. The ability of interstitial water flow to sustain substrate organisms and remove metabolic waste is thus reduced. These factors have significant implications for maintaining river habitat functions and point to the importance of continuing research on physical river processes. A review of the existing data on the critical shear stress for sediment movement indicated that the present knowledge needs to be expanded to provide design information for channel maintenance and management over a wide range of practical flow conditions. A sloping-pipe experimental facility was designed and constructed to research the effect of bed slope on the critical shear stress for initiation of sediment motion. Experimental work will begin in 98-99.

Articles/reports/conference presentations in FY 97/98

- (1) Liu, D., R.J. Maguire, Y.L. Lau, G.J. Pacepavicius, H. Okamura and I. Aoyama. Mercuric chloride-catalyzed hydrolysis of the new antifouling compound Irgarol 1051. *Water Research*. (submitted)
- (2) Lau, Y.L. and Krishnappan, B.G. 1997. Measurements of size distributions of settling flocs. NWRI contribution 97-223.
- (3) Lau, Y.L., D. Liu and G.J. Pacepavicius. 1998. Persistence of cyanazine in the aquatic environment. NWRI Contribution 98-005.
- (4) Lau, Y.L. and I.G. Droppo. 1998. Flume experiments with Hamilton Harbour coal pile deposits. NWRI Contribution No. 98-024.
- (5) Pacepavicius, G., Y.L. Lau, D. Liu, H. Okamura and I. Aoyama 1997. A rapid biochemical method for estimating biofilm mass. *Environ. Toxicol. Water Qual.* **12**: 97-100.
- (6) Liu, D., R.J. Maguire, Y.L. Lau, G.J. Pacepavicius, H. Okamura, and I. Aoyama. Biotransformation of Irgarol 1051. *Presented at the* Eighth International symposium on toxicity assessment. Perth, Australia, May 25-30, 1997.
- (7) Liu, D., G.J. Pacepavicius, R.J. Maguire, Y.L. Lau, H. Okamura and I. Aoyama. Review of cyclone fermentor test for assessing biodegradability. *Presented at the* Thirty-third Central Canadian Symposium on Water Pollution Research. Burlington, Ontario, February 9-10, 1998.

CURRENT RESEARCH, FY 97/98

PROJECT: Non-Point Sources of Pollution
STUDY TITLE: Characterisation of Physically-Unstable Aquatic Colloids Relevant to Flocculation
STUDY LEADER: G. G. Leppard

The first general scheme for classifying natural aquatic organic colloids was completed; the work leading up to this achievement was outlined in a recent encyclopaedia article. This work has attracted considerable attention, especially from the university and industrial sectors around the world. The rapid quantitative low-cost enumeration and characterisation technique for aquatic colloids was completed and published. Its use is encouraged in the Canadian industry.

In collaboration with I. Droppo (NWRI), Prof. S.N. Liss (Ryerson Polytechnic University) and Prof. D.G. Allen (University of Toronto) and the support of a new NSERC Strategic Grant, research is continuing on flocs and floc-producing colloids, especially as it relates to contaminant transport; sedimentation/resuspension processes; the engineered decontamination of water; and colloid-driven biogeochemical processes on a planetary scale. Colleagues from McMaster University, Agriculture and Agri-Food Canada and ten Canadian industries also contribute to the research team.

With previously acquired knowledge and state-of-the-art technology, the research team pursues the hypothesis that molecular-scale details of floc structure (especially colloidal structure not previously amenable to analysis) will unravel more information about floc processes in engineered water decontamination systems and major biogeochemical cycles. It is anticipated that this work will lead to improved decontamination technology and better management practices.

Articles/reports/conference presentations in FY 97/98

- (1) Leppard, G.G. and J. Buffle. 1998. Aquatic colloids and macromolecules: Effects on analysis. pp. 349-377. *In* R.A. Meyers (ed.) *The Encyclopedia of Environmental Analysis and Remediation*. John Wiley & Sons, New York.
- (2) Finlayson, J.C., B. Liao, I.G. Droppo, G.G. Leppard and S.N. Liss. 1998. The relationship between the structure of activated sludge flocs and the sorption of hydrophobic pollutants. *Water Sci. Technol.* (*in press*)
- (3) Bura, R., M. Cheung, B. Liao, J. Finlayson, B.C. Lee, I.G. Droppo, G.G. Leppard and S.N. Liss. 1998. Composition of extracellular polymeric substances in the activated sludge floc matrix. *Water Sci. Technol.* (*in press*)
- (4) Lienemann, C.-P., A. Heissenberger, G.G. Leppard and D. Perret. 1998. Optimal preparation of water samples for the examination of colloidal material by transmission electron microscopy. *Aquat. Microb. Ecol.* **14**: 205-213.

- (5) Leppard, G.G., M.M. West, D.T. Flannigan, J. Carson and J.N.A. Lott. 1997. A classification scheme for marine organic colloids in the Adriatic Sea: Colloid speciation by transmission electron microscopy. *Can. J. Fish. Aquat. Sci.* **54**: 2334-2349.
- (6) Droppo, I.G., G.G. Leppard, D.T. Flannigan and S.N. Liss. 1997. The freshwater floc: A functional relationship of water and organic and inorganic floc constituents affecting suspended sediment properties. *Water, Air & Soil Pollut.* **99**: 43-53.
- (7) Leppard, G.G. 1997. Colloidal organic fibrils of acid polysaccharides in surface waters: Electron-optical characteristics, activities and chemical estimates of abundance. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **120**(1-3): 1-15.
- (8) Jackson, T.A. and G.G. Leppard. 1997. Energy dispersive X-ray microanalysis: A powerful research technique of major importance to biogeochemistry and microbial ecology. *Presented at the 43rd International Conference on Analytical Sciences and Spectroscopy*, Montreal, PQ, Canada.
- (9) Flannigan, D.T., M. Cheung, J. Finlayson, B. Liao, I.G. Droppo, G.G. Leppard and S.N. Liss. Composition of extracellular polymeric substances in the activated sludge floc matrix. Abstr. *Presented at the 2nd Int. Conf. "Microorganisms in Activated Sludge and Biofilm Processes"*, Berkeley, California, USA, July 21-23, 1997.
- (10) Finlayson, J.C., B. Liao, I.G. Droppo, G.G. Leppard and S.N. Liss. The influence of organic contaminants on activated sludge floc properties. Abstr. *Presented at the 2nd Int. Conf. "Microorganisms in Activated Sludge and Biofilm Processes"*, Berkeley, California, USA, 21-23 July, 1997.
- (11) Leppard, G.G. Extracellular polymeric substances: Characterising their native associations by correlative microscopy. *Presented at the 47 Annual Conf. Canadian Society of Microbiologists*, Quebec City, PQ, June 15-19, 1997.
- (12) Leppard, G.G., D.T. Flannigan, D. Mavrocordatos, C.H. Marvin, D.W. Bryant and B.E. McCarry. Binding of polycyclic aromatic hydrocarbons by size classes of particulate in Hamilton Harbour water. *Presented at the 80th Annual Conf. & Exhib. Canadian Society for Chemistry*, Windsor, Ontario, June 1-5, 1997.
- (13) Leppard, G.G., I.G. Droppo and S.N. Liss. Microbial floc structure, adhesion and biofilm formation. *Presented at the Int. Conf. "Biofilms in Aquatic Systems"*, Warwick, United Kingdom, 13-16 April, 1997.

CURRENT RESEARCH, FY 97/98

PROJECT: Non-Point Sources of Pollution
STUDY TITLE: Agricultural Non-Point Sources of Pollution and Sustainable Agriculture
STUDY LEADER: H.Y.F. Ng,

Research was completed on the effects of controlled drainage/subirrigation on nitrate loss and crop yields. A multiyear collaborative study on baseline level of nitrogen in subsurface water was initiated. Work continues on the determination of nitrate leaching, phosphorus transport in soil columns and surface runoff at several experimental sites; this work is investigating the effects of water table control, subirrigation and tillage practices with respect to determining the agronomic and environmental benefits of best management practices (BMPs). Major findings are summarized below.

(1) Study of water table control and subirrigation technologies in support of BMPs

A study was conducted to evaluate the influence of water table control and subirrigation in two farm-plots of 1.9 ha each. One of the plots was equipped with a free drainage (FD) system, and the other one with a controlled drainage and subirrigation (CDS) system. Both plots had a sandy loam soil, were planted with corn, and were monitored from May to November 1996. The cumulative volume of drainage water from the CDS plot was larger (by 20%). The soil moisture content was higher (by 15%) in the CDS plot. The water table of the FD plot was 61% deeper below the soil surface, compared with the CDS plot. The cumulative nitrate loss in the FD plot was twice the amount of the CDS plot (291 kg and 146 kg respectively). The nitrate loading in rainwater accounted for 14% and 28%, respectively, of the cumulative nitrate losses in tile drainage water of the FD and CDS plots. The subirrigation water accounted for 1% (2 kg) of the nitrate loss in drainage water of the CDS plot. Flow weighted mean nitrate concentration in the drainage water was reduced from 19.1 mg/L in the FD plot to 10.8 mg/L in the CDS plot. The average rates of leaf transpiration and stomatal conductance, respectively, were 33% and 11% higher in the CDS plot than in the FD plot. The average corn yields were 11.0 t/ha in the CDS plot and 6.7 t/ha in the FD plot.

(2) Field monitoring of surface runoff and tile drainage water quality at experimental sites

In collaboration with the Harrow Research Station of Agriculture and Agri-Food Canada, water samples of surface runoff, tile drainage, piezometric wells and rain were collected from the Bicrel, Chevalier and Shanahan experimental sites. In addition, the micro-climate (air and soil temperatures, air humidity, wind speed and direction) near the soil surface was monitored at the Bicrel site.

The tile drainage water samples, collected from the three experimental sites in 1995 and 1996, were analyzed for total phosphorus (TP), filtered phosphorus (TFP), nitrate nitrogen (NO_3),

nitrite nitrogen (NO₂), potassium (K), cadmium(Cd), copper (Cu), and zinc (Zn) in controlled drainage plots (CD), controlled drainage/subirrigation plot (CDS), and free drainage plots (FD).

(3) Baseline levels of nitrogen in subsurface drainage water

An investigation of the baseline levels of nitrogen in subsurface drainage water was initiated in order to quantify the efficiency of nitrogen use. A rainwater collector was installed to complement the field monitoring program. Because of a very dry summer, rainwater and tile drainage samples were only collected from September onward. The samples were submitted for analysis of Cl, K, Na, NO₃, and TP. The preliminary results were used to compare background nitrogen concentration levels of the field plots. The plots have been under grass cover and without input of nitrogen by fertilizer or manure for two years before the initiation of the study. This study is done in collaboration with the School of Engineering, University of Guelph.

Articles/reports/conference presentations in FY 97/98

- (1) Al-Abed, N., H.Y.F. Ng, C.S. Tan, C.F. Drury, and J.D. Gaynor. 1997. Modelling of Nitrate in a Tiled-Drained Field Using RZWQM. NWRI Contribution No. 97-187.
- (2) Ng, H.Y.F. Agrochemical Transport from a Small Agricultural Watershed. *Presented at the IAGLR '97. IAGLR 40th Conference*, Buffalo State College & University of Buffalo, Buffalo, N.Y., June 1-5, 1997.
- (3) Ng, H.Y.F., C.F. Drury, V.K. Serem, C.S. Tan, and J.D. Gaynor. Modelling and Testing of the Effect of Combined Tillage, Cropping and Water Management Practices on Nitrate Leaching in Clay Soil. *J. Agri. Water Mgmt.* (submitted)
- (4) Ng, H.Y.F., Al-Abed, N., C.S. Tan, C.F. Drury, and J.D. Gaynor. Modelling nitrate losses in a tile drainage field using RZWQM. *Presented at the World Water Congress*, Montreal, September 1-6, 1997.
- (5) Tan, C.S., C.F. Drury, M. Soultani, I.J. Van Wesenbeeck, H.Y.F. Ng, J.D. Gaynor, and T.W. Welacky. Effect of Controlled Drainage on Soybean Yield and Water Quality Under No-Tillage and Conventional Tillage Systems at the Field Scale. *Presented at the 1997 ASAE Annual International Meeting*, Minneapolis, Minnesota, August 10-14, 1997.
- (6) Tan, C.S., C.F. Drury, M. Soultani, I.J. Van Wesenbeeck, H.Y.F. Ng, J.D. Gaynor, and T.W. Welacky. Effect of controlled drainage/subirrigation on tile drainage water quality and crop yields at the field scale. On-Farm Research Program of the Canada-Ontario Green Plan. (submitted)
- (7) Tan, C.S., C.F. Drury, M. Soultani, I.J. Van Wesenbeeck, H.Y.F. Ng, J.D. Gaynor, and T.W. Welacky. 1997. Effect of controlled drainage/subirrigation on tomato yield and water quality. pp. 327-333. *In K.S. Chartzoulakis (ed) Proc. 2nd Int. Sym. On Irrigation of Hort. Crops. Acta. Hort.* 449 Vol. 1. ISHS.

- (8) Tan, C.S., C.F. Drury, M. Soultani, I.J. Van Wesenbeeck, H.Y.F. Ng, J.D. Gaynor, and T.W. Welacky. Effect of Controlled Drainage and Subirrigation on Tomato Yield and Water Quality at the Field Scale. *Presented at the Seventh International Drainage Symposium, Hyatt Orlando, Orlando, Florida, March 8-11, 1998.*

CURRENT RESEARCH, FY 97/98

PRIORITY SUBSTANCES - EXPOSURE/EFFECTS AQUATIC ECOSYSTEM PROTECTION BRANCH

The project develops and applies techniques to screen and assess priority substances and effluents for their potential to cause effects on aquatic biota. In collaboration with other researchers, methods are developed to isolate, identify and characterize chemicals responsible for physiological and reproductive impacts. The project has the capabilities to measure and predict exposure to and effects of a wide variety of contaminants in support of various national and regional ecosystem programs, e.g., CEPA, TSMP, EEM, Great Lakes 2000, etc.

CURRENT RESEARCH, FY 97/98

PROJECT: Priority Substances - Exposure/Effects
STUDY TITLE: Environmental Chemistry and Effects of Priority Substances
STUDY LEADER: M.R. Servos

The assessment of the exposure of priority substances and their ability to cause impacts on the survival, growth, development and reproduction of biota is the primary focus of this study. Techniques to screen priority chemicals, effluents and environmental samples for developmental and reproductive impairment as well as techniques to isolate and identify chemicals responsible for biological impacts are developed and applied. This includes: the development and application of bioassays (partial to full life-cycle tests) for chronic, reproductive and developmental effects on biota exposed to toxic chemicals (municipal, textile effluents, nonylphenol ethoxylates, pesticides); determination of exposure and effects of natural and synthetic hormones in municipal effluents; the examination of remedial options to reduce the release of toxic and estrogenic chemicals from priority substances and effluents; and the role of food web structure and function on controlling the biomagnification of organic contaminants.

(1) The exposure and effects of endocrine disrupting chemicals in municipal effluents

The development and validation of methods to measure the concentrations of natural and synthetic hormones in effluent and receiving waters was completed and applied to determine the distribution of these chemicals entering the environment. Samples collected at ten municipal sewage treatment plants indicate that natural and synthetic hormones are detectable in Canadian effluents in the low ng/L range, similar to that observed in other countries. A collaborative research program was initiated with the Water Technologies International, the Burlington Environmental Technology Office, and local municipalities to assess the fate and distribution of nonylphenols (PSL-2), natural and synthetic hormones in a municipal treatment plant which also received textile mill effluents. The fate of the chemicals was determined at each of the major process steps in the treatment plant including UV-disinfection and final effluent. A study on the fate and effects of the effluent in the receiving water was also underway during the same period to evaluate the exposure and potential impacts on fish. Laboratory experiments established that the effluent has the potential to cause endocrine disruption in fish. The study has been expanded to a national survey of the distribution and effects of these chemicals in municipal effluents across Canada.

(2) Exposure and effects of trace contaminants in lampricide formulations

Batches of 3-trifluoromethyl-4-nitrophenol (TFM) were tested for mixed function oxidase (MFO) induction and trace contaminants in support of the Great Lakes Fishery Commission Sea Lamprey Control Programs. Contamination of TFM formulations, fish and sediment collected during a treatment of the Bad River, Wisconsin was completed as part of an agreement with the Bad River Tribal Council and the US-Fish and Wildlife Service. Toxicity identification evaluations of the

formulations were conducted to isolate and to identify the chemicals responsible for both MFO induction and estrogenic responses. Studies of the temporal and spatial distribution of biological responses during regular treatments of Bronte Creek and Lynde Creek were completed.

(3) Polychlorinated dioxins and furans in pulp and paper mill effluents

A study was completed on the fate and distribution of polychlorinated dioxins in fish exposed to effluents of pulp and paper mill which have undergone treatment and process changes. This data played a critical role in the interpretation of data from Cycle one of the Environmental Effects Monitoring (EEM) program. The data clearly demonstrated a dramatic response of the environment to the control and release of dioxins from these effluents.

(4) Using stable isotopes to understand complex food webs

Several studies on Lake Ontario and Erie to understand the energy flow and bioaccumulation of contaminants in food webs were completed. Stable isotopes have proven to be very useful to understand the patterns of contamination in the food webs in the lakes, including top predators such as lake trout. The seasonal dynamics of the system, especially at the lower end of the food chain, is important in controlling the isotopic signatures in higher organisms. Careful examination of isotopic signature is a very effective tool to understand the interactions of organisms in these complex ecosystems. This work continues to contribute to our understanding of the structure and function of large lakes and increases our ability to predict the bioaccumulation of toxic chemicals in complex food webs.

Articles/reports/conference presentations in FY 97/98

- (1) Chambers, P.A., M. Allard, S.L. Walker, J. Marsalek, J. Lawrence, M. Servos, J. Busnarda, K.S. Munger, K. Adare, C. Jefferson, R.A. Kent and M.P. Wong. 1997. Impacts of municipal wastewater effluents on Canadian waters: A Review. *Water Qual. Res. J. Canada* 32(4): 659-713.
- (2) Hewitt, L.M., J.H. Carey, K.R. Munkittrick, J.L. Parrott, K.R. Solomon and M.R. Servos. Identification of chloro-nitro-trifluoromethyl-substituted dibenzo-*p*-dioxins in lampricide formulation of 3-trifluoromethyl-4-nitrophenol (TFM): Assessment to induce MFO activity. *Env. Toxicol. Chem.*, *in press*
- (3) Hewitt, M.R., I. Scott, K. Munkittrick, K. Solomon and M.R. Servos. 1997. Hepatic MFO activity and vitellogenin induction in fish following a treatment of lampricide 3-trifluoromethyl-4-nitrophenol (TFM). *Can. J. Fisheries and Aquatic Sciences*, *in press*
- (4) Leggett, M.F., M.R. Servos, R. Hesslein, O. Johannsson and D.G. Dixon. Influence of inorganic nitrogen cycling on the ¹⁵N of Lake Ontario biota. *Oecologia*. (*submitted*)
- (5) Leggett, M.F., M.R. Servos, R. Hesslein, O. Johannsson, E.S. Millard and D.G. Dixon. Biochemical influences on the carbon isotopic signatures of Lake Ontario biota. *Oecologia*. (*submitted*)

- (6) Munkittrick, K.R., M.E. McMaster, L.H. McCarthy, M.R. Servos and G.J. Van Der Kraak. An overview of recent studies on the potential of pulp and paper mill effluents to impact reproductive function in fish. *J. Toxicol. Environ. Health*, *in press*
- (7) Munkittrick, K.R., M.E. McMaster, C. Portt, W.N. Gibbons, A. Farwell, L. Ruemper, M.R. Servos, J. Nickle and G.J. Van Der Kraak. The development of cumulative effects assessment tools using fish populations. *In Critical Methodologies for the Study of Ecosystem Health*, University of California, Davis, CA., *in press*
- (8) Munkittrick, K.R., M.E. McMaster, M.R. Servos and G.J. Van Der Kraak. Changes in the reproductive performance of fish in Jackfish Bay over the period of mill modernization. *Presented at the 3rd Internat. Conf. Environmental Fate and Effects of Pulp and Paper Mill Effluents*, Rotorua, NZ, November 9-13, 1997.
- (9) Munkittrick, K.M., S.R. Megraw, A. Colodey, S. Luce, S. Courtenay, M. Paine, M. Servos, M. Spafford, C. Langlois, P. Martel and C. Levings. Final Report of the Fish Survey Expert Working Group for EEM cycle one. EEM/1997/6.
- (10) Munkittrick, K.R., O. Sandstrom, A. Larsson, G.J. Van Der Kraak, L. Forlin, E. Lindesjoo and M.R. Servos. A reassessment of the original reviews of Norrsundet and Jackfish Bay field studies (a retrospective look at risk assessments). *Presented at the 3rd Internat. Conf. Environmental Fate and Effects of Pulp and Paper Mill Effluents*, Rotorua, NZ, November 9-13, 1997.
- (11) Munkittrick, K.R., M.R. Servos, K. Gorman, B. Blunt, M.E. McMaster, and G.J. Van Der Kraak. 1997. Characteristics of EROD induction associated with exposure to pulp mill effluent. Rao et al. *Methods in Ecotoxicology* (*submitted*)
- (12) Parrott, J., M.R. van den Heuvel, L.M. Hewitt, M.R. Servos, M.A. Baker and K.R. Munkittrick. Isolation of MFO inducers from tissues of white suckers caged in kraft mill effluent. *Presented at the 3rd Internat. Conf. Environmental Fate and Effects of Pulp and Paper Mill Effluents*, Rotorua, NZ, November 9-13, 1997.
- (13) Rochfort, Q., I. Scott, R. McInnis, A. Jurkovic, J. Marsalek and M. Servos. 1998. Chronic toxicity of high and low intensity UV disinfected effluents from selected sewage treatment plants in Ontario: NWRI Contribution No. 98-015.
- (14) Servos, M.R. and S. Luce (eds). 1997. *Proceedings of the Environment Canada Workshop on Endocrine Disruptor Compounds: Identifying Research Needs and Priorities*. Niagara Falls, Ontario, Oct. 23-24, 1997.
- (15) Servos, M.R., S. Luce, J. Toito, S. Huestis, M. McMaster and K.R. Munkittrick. The rapid decline of polychlorinated dibenzo-*p*-dioxins and furans in fish exposed to pulp and paper mill effluents in Canada. *Presented at the 3rd Internat. Conf. Environmental Fate and Effects of Pulp and Paper Mill Effluents*, Rotorua, NZ, November 9-13, 1997.
- (16) Servos, M.R., C. Luthe, A. Colodey, W. Fairchild, M. Ikonomou, S. Luce, D. Sergeant and S. Walker. Dioxin Expert Working Group final report of the Environmental Effects Monitoring Program. Environment Canada EEM/1997/3.

- (17) Servos, M.R., I. Scott, M. Hewitt and J. Toito. 1998. TFM Formulation Related Contaminants in Fish and Sediments From the Bad River. NWRI Contribution No. 98-016
- (18) Whyte, J.J., M.J. vanden Heuvel, J.H. Clemons, M.R. Servos, D.G. Dixon and N. Bols. 1997. Comparison of mammalian and teleost cell line bioassay and chemically derived TCDD-equivalent concentrations in hepatic tissue of lake trout from Lake Superior and Lake Ontario.
- (19) Fawell, A., K.R. Munkittrick, M.R. Servos and K. Solomon. The use of carbon and nitrogen stable isotopes to characterize hydroelectric development in river systems. *Presented at the 18th Annual Meeting Soc. Environ. Toxicol. Chem., San Francisco, CA November 16-20, 1997.*
- (20) Hewitt, L.M., J. Carey, K.R. Munkittrick, J. Parrott, K. Solomon and M. Servos. 1997. Confirmation of chloro-nitro-trifluormethyl substituted dibenzo-*p*-dioxins in lampricide formulations of 3-trifluoromethyl-4-nitrophenol (TFM): assessment to induce P450 1A1 activity. *Presented at the 18th Annual Meeting Soc. Environ. Toxicol. Chem., San Francisco, CA November 16-20, 1997.*
- (21) Leggett, M.F., M. R. Servos, R. Hesslein, S. Millard and D.G. Dixon. The relationship between the rate of primary production and the carbon isotopic composition of particulate matter in Lake Ontario. *Presented at the International Association of Great Lakes Research Annual Conference, Buffalo, N.Y., June 1-5, 1997.*
- (22) Luce, S., Munkittrick, K.M., S.R. Megraw, A. Colodey, S. Courtenay, M. Paine, M. Servos, M. Spafford, C. Langlois, P. Martel and C. Levings. EEM fish survey: a national perspective and transition to cycle 2. *Presented at the 24th Annual Aquatic Toxicity Workshop. Niagara Falls, Ontario, Oct 19-22, 1997.*
- (23) Rochfort, Q. I. Scott, R. McInnis, A. Jurkovic, J. Toito, M. Servos and J. Marsalek. Chronic toxicity of high and low intensity uv disinfected effluents from selected sewage treatment plants in Ontario. *Presented at the 24th Annual Aquatic Toxicity Workshop. Niagara Falls, Ontario, Oct 19-22, 1997.*
- (24) Servos, M.R. Historical and current levels of dioxins at pulp and paper mill sites. *Presented at the Pulp and Paper EEM Workshop, Montreal Dec. 2-3, 1997.*
- (25) Servos, M.R. The emerging issue of endocrine disruptors, the Canadian perspective. *Presented to the Dept. of Environmental Chemistry, Masaryk University, Brno, Czech Republic, Feb. 19, 1998.*
- (26) Servos, M., C. Luthe, A. Colodey, W. Fairchild, M. Ikononou, S. Luce, D. Sergeant, and S. Walker. EEM - Transition to cycle two: dioxins and furans recommendations. *Presented at the 24th Annual Aquatic Toxicity Workshop. Niagara Falls, Ontario, Oct 19-22, 1997.*

- (27) Van Der Kraak, G., L. Tremblay, K. Wells, D. Jans, T. Zacharewski, E.J. Squires, M. McMaster, M. Servos, M. Hewitt and K. Munkittrick. Validation of reproductive endocrine biomarkers across different levels of biological complexity. *Presented at the Canadian Network of Toxicology Centres Annual Research Symposia, Toronto, Ontario, March 23-25, 1998.*

CURRENT RESEARCH, FY 97/98

PROJECT: Priority Substances - Exposure/Effects
STUDY TITLE: Reproductive Toxicology, Endocrinology
STUDY LEADER: Scott Brown

This project investigates the toxicological mechanisms of deleterious substances on fish growth, reproduction and development. The work contributes knowledge towards understanding the effects of priority substances in support of TSMP, CEPA (PSL 1 and 2) and Great Lakes initiatives (Great Lakes 2000, COA). 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.

(1) Chemically-induced reproductive disturbances in fishes from the Great Lakes.

Recently, salmonid species in the Great Lakes Basin have experienced a post-hatch mortality as high as 100 %. Reproductive failure due to this 'Early Mortality Syndrome' (EMS) is a major concern because it may represent a serious impediment to the restoration of sustainable populations of lake trout and Atlantic salmon in the lower Great Lakes. The work has international ramifications because, in the U.S., there have been large-scale losses of hatchery-reared salmonids due to EMS and, in Scandinavia, there are similar urgent concerns regarding stocks of Baltic Sea salmon and trout. Project work has described the role of oxidative stress and antioxidants in EMS (see references 1 and 2). The levels of micronutrients were described in parental diets (reference 5) and epidemiological work clearly links the presence of dietary alewife as an important factor contributing to thiamine deficiency in adults (reference 3) and in developing larvae exhibiting EMS (reference 4).

(2) Exposure to priority substances and effluents - role of organochlorine contaminants in mechanisms controlling growth and development in fish

Elevated concentrations of different organochlorines have been noted in fish from many regions. However few studies have investigated their biological effects that would be suitable to set targets for 'virtual elimination'. The project evaluated some possible mechanisms whereby organochlorines may disrupt the known actions of thyroid hormones and antioxidant vitamins. Knowledge of these processes could lead to the development of new and specific biomarkers of

organochlorine toxicity in fishes. 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 biomarkers of adverse effects. The metabolites as well as the lipophilic contaminants may be parentally transferred to offspring and create deleterious effects on early life stages. Impaired thyroid function were described in fish exposed to co-planar PCBs (see reference 7) and antioxidant deficiencies were described for fish exposed to pulp mill effluents (see reference 8).

Articles/reports/conference presentations in FY 1997/98

- (1) Palace, V.P., S.B. Brown, C.L. Baron, J. Fitzsimons, B. Woodin, J.J. Stegeman and J.F. Klaverkamp. An assessment of the relationships among oxidative stress, the antioxidant vitamins and early mortality syndrome (EMS) of lake trout (*Salvelinus namaycush*) from Lake Ontario. *Aquat. Toxicol.*, *in press*
- (2) Palace, V.P., S.B. Brown, C.L. Baron, J. Fitzsimons and J.F. Klaverkamp. Relationships between induction of the Phase I enzyme system and oxidative stress: relevance for lake trout from Lake Ontario and early mortality syndrome (EMS) of their offspring. *In* G. McDonald, J. Fitzsimons and D.C. Honeyfield (eds.) *Early Life Stage Mortality Syndrome in Fishes of the Great Lakes and the Baltic Sea*. American Fisheries Society, Bethesda, MD, *in press*
- (3) Fisher, J.P., S.B. Brown, S. Connelly, T. Chiotte and C.C. Kruger. Interspecies comparison of whole blood thiamine in salmonids from the Finger Lakes, and effect of maternal size. *In* G. McDonald, J. Fitzsimons and D.C. Honeyfield (eds.) *Early Life Stage Mortality Syndrome in Fishes of the Great Lakes and the Baltic Sea*. American Fisheries Society, Bethesda, MD., *in press*
- (4) Fitzsimons, J. D., and S.B. Brown. Depressed egg thiamine levels in Great Lakes lake trout and their relationship with diet. *In* G. McDonald, J. Fitzsimons and D.C. Honeyfield (eds.) *Early Life Stage Mortality Syndrome in Fishes of the Great Lakes and the Baltic Sea*. American Fisheries Society, Bethesda, MD., *in press*
- (5) Fitzsimons, J. D., S.B. Brown and L. Vandenbyllaardt. Thiamine levels in food chains of the Great Lakes. *In* G. McDonald, J. Fitzsimons and D.C. Honeyfield (eds.) *Early Life Stage Mortality Syndrome in Fishes of the Great Lakes and the Baltic Sea*. American Fisheries Society, Bethesda, MD., *in press*
- (6) Honeyfield, D., J. Fitzsimons, S.B. Brown, S. Marcquenski and D. McDonald. Introduction: early life stage disorders in fishes of the Great Lakes and the Baltic. *In* G. McDonald, J. Fitzsimons and D.C. Honeyfield (eds.) *Early Life Stage Mortality Syndrome in Fishes of the Great Lakes and the Baltic Sea*. American Fisheries Society, Bethesda, MD., *in press*
- (7) Brown, S.B, R. E. Evans, K. W. Finnson, and L. Vandenbyllaardt. Thyroid function in lake trout (*Salvelinus namaycush*) exposed to coplanar 3,3',4,4',5-pentachlorobiphenyl. *Presented at the 24th Annual Aquatic Toxicity Workshop*, Niagara Falls, Ontario, October 20-22, 1997.

- (8) Brown, S. B., K. Munkittrick, C. Bezte, W.L. Lockhart and L. Vandenbyllardt. Retinoid status in fish exposed to pulp mill effluent. *Presented at the SETAC 18th Annual Meeting Conference, San Francisco, November 16-20, 1997.*

CURRENT RESEARCH, FY 97/98

PROJECT: Priority Substances - Exposure/Effects
STUDY TITLE: Xenoestrogenic Screening Procedure to Identify Substances of Concern in the Great Lakes Ecosystem
STUDY LEADER: B.K. Burnison

During the past few years, extensive research programs were initiated to determine the presence of man-made chemicals which were capable of producing endocrine disrupting effects. Estrogenic effects (i.e., feminisation) were emphasized 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 17 β -estradiol and compete for the binding to the estrogen receptor. In order to quickly measure the estrogenicity of an effluent, we need to use a laboratory screening procedure such as the yeast estrogen system (YES). Naturally, yeast do not contain sex steroid or thyroid hormone receptors. However, recombinant yeast strains have been developed which have the human estrogen receptor inserted (transfected) into the yeast genome. Research was initiated this year to transfer the yeast estrogen screen (YES) technology into our laboratory for the purpose of determining estrogenic effects in Canadian municipal sewage effluents.

(1) YES bioassay

We have successfully transferred the yeast estrogen screen (YES) technology into our laboratory. The dose-response data for 17 β -estradiol, estrone, ethinyl estradiol, octyl phenol, and nonyl phenol ('standard suite') were very similar to literature values (i.e., estradiol EC₅₀ = 2.2×10^{-10} M). We have also tested DDT (EC₅₀ = 1.1×10^{-6} M), atrazine (no induction in the range of 0.01 and 1 ppm), dibutyl phthalate (EC₅₀ = 3.7×10^{-3} M) and retene (some estrogenic activity at extremely high concentrations, possible contaminant). We have done a small survey of five municipal sewage effluents and found that even with filter sterilisation and keeping the samples at 4°C, there was a change in estrogenic activity in the whole effluent during a week's storage. We have also set-up the competitive radioligand binding procedure to verify the estrogenic activities noted by the YES bioassay. The radioligand binding utilises tritiated estradiol and the human estrogen receptor isolated from transformed yeast cells.

(2) Municipal effluent fractionation

The Toxicity Identification and Evaluation (TIE) approach will be used to isolate and identify estrogenic compounds in municipal sewage effluents. Chemical fractionation will be monitored, using the YES bioassay, to find active components. Municipal sewage effluent (4L) was used for preliminary experiments on the isolation of estrogenic substances. The effluent was either

processed through solid phase extraction (SPE) columns or Envi-C18 disks after glass fibre filtration. The adsorbed substances were sequentially eluted with various concentrations of methanol, followed by DCM and hexane. The eluents were dried with nitrogen and analysed with the YES bioassay. The estrogenic activity usually eluted off the SPEs at the same concentration of methanol that eluted the estradiol standard (i.e., 75% methanol). If Envi-C18 disks are used, narrow 'cuts' with various concentrations of methanol can not be used. We did a 25% methanol wash and then eluted with 100% methanol before more non-polar solvents were used. Therefore, the estrogenic activity was in the 100% methanol eluent. We have also developed a HPLC procedure that we can get baseline separation for the six 'standard suite' estrogenic compounds.

We have been concerned about the contribution of estrogen conjugates (glucuronides and sulphates) to the YES bioassay results and the fate of these conjugates in the sewage effluent. We have added the liver microsomal S9 fraction to samples and we have observed an increase in estrogenic activity. We are currently investigating the use of the specific enzymes (glucuronidase and sulfatase) to detect the presence of these conjugates.

Articles/reports/conference presentations in FY 1997-98

- (1) Burnison, B.K. Review of Bioconcentration, Bioaccumulation and K_{ow} Techniques. *Water Qual. Res. J. Canada., in press*
- (2) Burnison, B.K., M.E. Comba, J.H. Carey and J. Parrott. Isolation and tentative identification of the compound responsible for fish MFO-induction in BKME. *Presented at the 18th Annual Meeting of the Society of Environmental Toxicology and Chemistry, San Francisco, CA, 16-20 November, 1997.*
- (3) Burnison B.K. and D.J. Nuttley. Fluorescence quenching of PAHs: Potential experimental artifacts during the determination of K_{doc} values. *Presented at the 24th Annual Aquatic Toxicity Workshop: Niagara Falls, ON, 20-22 October, 1997.*
- (4) Richards, J., B.K. Burnison and R.C. Playle. 1997. Are all dissolved organic matters equally protective against bindings in fish gills. *Presented at the 24th Annual Aquatic Toxicity Workshop: Niagara Falls, ON, 20-22 October, 1997.*
- (5) Burnison, B.K. 1997. Review of bioconcentration, bioaccumulation and K_{ow} techniques for the purpose of developing regulations under CEPA. NWRI Contribution No. 98-011.
- (6) Rose-Janes, N., J. Richards, L. Ostrowski, K. Burnison and R. Playle. Protective effects of dissolved organic matter against the physiological disturbances of waterbourne silver on rainbow trout. *Presented at the 5th International Argentum Conference on the "Transport, Fate and Effects of Silver in the Environment", Hamilton, ON, Sept.28-Oct. 1, 1997.*
- (7) Richards, J., N. Rose-Janes, L. Ostrowski, B.K. Burnison and R.C. Playle. Are all dissolved organic matters equally protective against bindings in fish gills. *Presented at the 5th International Argentum Conference on the "Transport, Fate and Effects of Silver in the Environment", Hamilton, ON, Sept.28-Oct. 1, 1997.*

CURRENT RESEARCH, FY 97/98

PROJECT: Priority Substances - Exposure/Effects

STUDY TITLE: Development and Application of Quantitative Structure-Activity Relationships to Acute, Chronic, Reproductive and Developmental Data/Endpoints.

STUDY LEADER: K.L.E. Kaiser

Research proceeded on the analysis of Microtox and fathead minnow toxicity data using neural network algorithms. In earlier work, a set of 419 individual chemicals from diverse classes of chemicals were analyzed to elucidate single linear and multiple linear relationships between their physico-chemical bulk parameters and molecular descriptors. The results of this work were presented at the QSAR 1996 workshop in Denmark with the proceedings published in early 1998.

A considerable part of time involved the collation, extraction and analysis of literature data on the biodegradability of substances with different tests and their applicability to predict environmental degradation rates and/or half lives. While all available evidence suggests that there are no clear correlations between such biodegradability tests and environmental degradation rates, this work, undertaken on special commission for the Commercial Chemicals Evaluation Branch, clearly led to some recommendations on the usefulness and applicability of some of the available testing methods in this field. Figure 1 gives a schematic representation of a typical biodegradation curve for a moderately persistent substance.

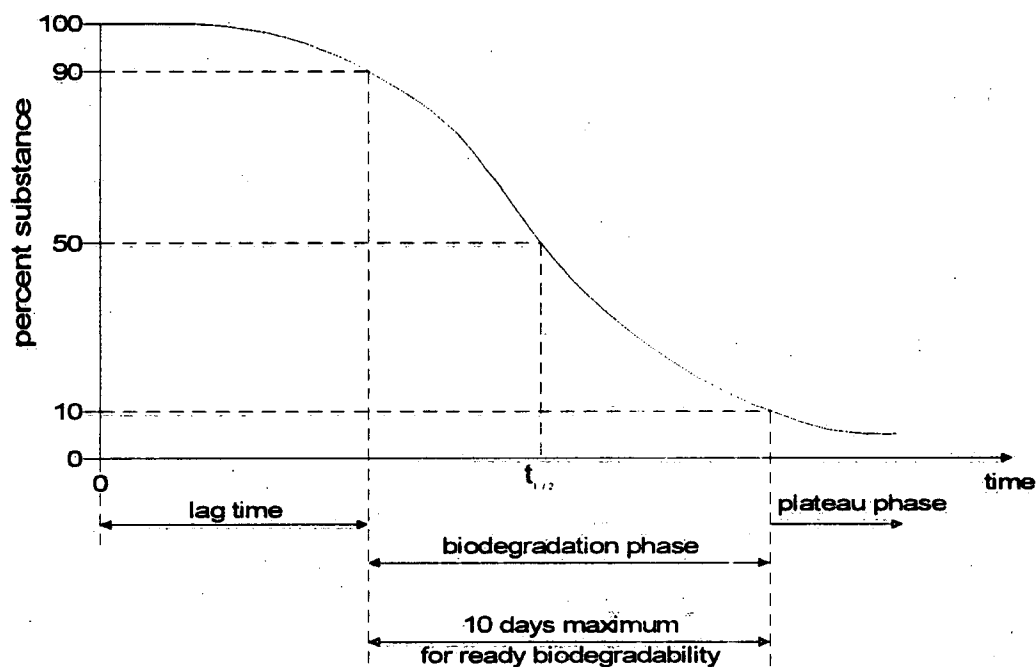


Fig. 1. Schematic diagram of a typical biodegradation process of a moderately persistent substance

Following the QSAR '96 workshop, agreement was reached with a research group in Germany (Dr. G. Schüürmann, UFZ, Leipzig, Germany) to jointly study this dataset with other mathematical means. Specifically, it was agreed that the UFZ group would investigate the use of principal component analysis to develop models for these data. The data were sent to UFZ and some intermediate modeling results have been returned since from there, but no final analysis has yet emerged. In contrast, we proceeded to study the data set with feed forward backpropagation and probabilistic neural network models. The results of this work has been quite encouraging and various aspects of the study have been presented at the SETAC conference (Nov. 97) and published in two articles in the literature.

Work in the laboratory continued with preparation and processing of fish and water samples from several sources. For example, water samples were analyzed for several pulp mill related contaminants, such as retene, dichloroguaiacol and trichloroveratrole in support of research funded by the Fraser River Action Plan. Fish samples were analyzed for organochlorine contaminants in support of research on improving environmental contaminant fate models. In collaboration with Canadian Museum of Nature scientist C. Renaud, work continued on the analysis of historical lamprey ammocoete samples from some Lake Superior tributaries. Only a few additional chemicals were measured with the Microtox analyzer to determine their acute toxicity in support of CEPA, GL2000, QSAR and emerging issues. In addition, toxicity determination were made for a number water samples in support of groundwater degradation testing experiments.

Articles/reports/conference presentations in FY 1997-98

- (1) Kaiser, K.L.E., S.P. Niculescu and G. Schüürmann. 1997. Feed forward backpropagation neural networks and their use in predicting the acute toxicity of chemicals to the fathead minnow. *Water Qual. Res. J. Canada* **32**: 637-657.
- (2) Kaiser, K.L.E., S.P. Niculescu and G. Schüürmann. Probabilistic neural network modeling of the acute toxicity of chemicals to the fathead minnow and *Vibrio fischeri* bacteria. *Presented at the Society of Environmental Toxicology and Chemistry, 18th Annual Meeting, San Francisco, November 1997.*
- (3) Niculescu, S.P., K.L.E. Kaiser and G. Schüürmann. 1998. Influence of data pre-processing and kernel selection on probabilistic neural network modelling of the acute toxicity of chemicals to the fathead minnow and *Vibrio fischeri* bacteria. *Water Qual. Res. J. Canada* **33**: 153-165.
- (4) Kaiser, K.L.E. 1997. Review of biodegradability tests for the purpose of developing regulations under CEPA. NWRI Contribution No. 97-213.
- (5) Kaiser, K.L.E., S.P. Niculescu and M.B. McKinnon. 1998. On the simple linear regression, the multiple linear regression and the elementary probabilistic neural network with Gaussian kernel's performance in modeling toxicity values to fathead minnow based on Microtox data, the octanol/water partition coefficient and various structural descriptors for a 419 compound data set. pp. 285-297. *In* F. Chen and G. Schüürmann (eds.) *QSAR in Environmental Sciences - VII*, SETAC Press, Pensacola, FL.

- (6) Kaiser, K.L.E. and S. Ponton. 1997. Water Quality Research Journal of Canada. Web page at <http://www.cciw.ca/wqrjc/wqrjce.htm> and <http://www.cciw.ca/wqrjc/wqrjcf.htm> and derivatives thereof.

CURRENT RESEARCH - FY 97/98

PROJECT: Priority Substances - Exposure/Effects
STUDY TITLE: Fate and Effects of Contaminants on the Aquatic Ecosystems
STUDY LEADER: T. Mayer

Research was completed on the identification of nutrient sources to Point Pelee Marsh ecosystem and new research was initiated on the toxicity of the highway runoff. Recent progress is summarized below:

(1) Nutrient sources in freshwater wetlands

The results of a multidisciplinary investigation on the identification of nutrient sources at Point Pelee have been compiled and synthesized. Two areas (Sanctuary Pond and Blue Heron) in the Point Pelee National Park, Ontario were investigated. At Blue Heron, the sediment phosphorus, n-alkane data and the distribution of coprostanol, a sewage specific marker, suggest anthropogenic input of phosphorus to the marsh. At the Sanctuary Pond, high nutrient concentrations were found in sediment porewater. The orthophosphate (PO_4) and ammonia (NH_3) concentrations (3 mg/L and 20 mg/L, respectively) were substantially higher than those observed in the porewater of Lake Erie sediments, and were comparable to the porewater concentrations of sediments from hyper-eutrophic lakes or wetlands. The levels of dissolved PO_4 produced by the decomposition of sediment organic matter are governed by phosphate mineral equilibria. Geochemical modelling indicates that porewater is highly supersaturated with respect to the Ca mineral phase, hydroxyapatite and to the Fe mineral phase vivianite and less supersaturated with respect to the Al mineral phase, variscite. No differences in the porewater profiles nor in the levels of dissolved nutrients were observed between the two investigated sites. Likewise, there were no spatial differences in the levels of phosphorus in sediments. The lack of spatial differences in nutrient concentrations and the physical mixing of sediments by carp, introducing the nutrient-rich porewater into the water column, suggest that benthic sediments are responsible for excessive nutrient inputs to the water column and subsequent deterioration of water quality.

(2) Toxicity of highway runoff

The investigation of toxicity of highway runoff was expanded and two new sampling sites were added to the sampling network. The aim of the study is to investigate the effect of the volume and type of traffic on the levels of contaminants in highway runoff and their toxicity to aquatic organisms. Seasonal aspects of toxicity of runoff are also evaluated. Preliminary results indicate that highway runoff containing high concentrations of road salt from winter road maintenance is acutely toxic to *Daphnia magna*. No acute toxicity of whole water runoff was observed at other times. The results of the submitochondria (SMP) toxicity tests are consistent with the *Daphnia*

magna acute toxicity tests. Road salts are among the substances recommended for assessment under CEPA's second Priority Substances List (PSL2).

In order to assess changes in toxicity and composition of highway runoff, time series of runoff samples were collected from a rain event. Based on earlier results, composite samples from the first flush and subsequent short time intervals were used for the toxicity tests. Sublethal tests, such as reproductive impairment to *Ceriodaphnia dubia* were used to assess toxicity. A sharp decline in toxicity occurred over time during the precipitation event, i.e., a decline in mortality of *C. dubia* and an increase in the number of their neonates. The runoff samples are presently being analyzed for a variety of contaminants, including heavy metals, polycyclic aromatic hydrocarbons (PAHs) and chlorides.

Articles/reports/conference presentations in FY 1997/98

- (1) Marsalek, J., B. Brownlee, T. Mayer, S. Laval and G. A. Larkin. 1997. Heavy metals and PAHs in stormwater runoff from the Skyway Bridge, Burlington, Ontario. *Water Qual. Res. J. Canada* 32(4): 815-827.
- (2) Mayer, T., C. Ptacek and L. Zanini. 1997. The role of sediments in nutrient dynamics in hyper-eutrophic marshes of Point Pelee, Ontario, Canada. NWRI Contribution No. 97-212.
- (3) Mayer, T., R.A. Bourbonniere and A. Crowe. 1997. Assessment of potential septic system-derived phosphorus input to the Point Pelee Marsh. NWRI Contribution No. 98-012.
- (4) Mayer, T. and L. Zanini. The role of sediments in nutrient dynamics in hyper-eutrophic marshes of Point Pelee, Ontario, Canada. *Presented at the 17th International Symposium of the North American Lake Management Society, Houston, Texas, December 2 - 6, 1997.*
- (5) Mayer, T., L. Zanini, R.A. Bourbonniere and S. Telford. Investigation of nutrient sources to Point Pelee Marsh. *Presented at the Second Annual Meeting of Parks Research Forum for Ontario, Peterborough, Ontario, February 5 - 6, 1998.*
- (6) Crowe, A.S., C.J. Ptacek, R. McCrea, J. Coakley, T. Mayer, P.A. Huddart, D. Thompson and S. Shikaze. An overview of Environment Canada's Groundwater research and monitoring activities at Point Pelee National Park, Ontario. *Presented at the Second Annual Meeting of Parks Research Forum for Ontario, Peterborough, Ontario, February 5 - 6, 1998.*

CURRENT RESEARCH, FY 97-98

PROJECT: Priority Substances - Exposure/Effects
STUDY TITLE: 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

Studies to compare caged and native mussels as biomonitors of organic contaminants in the St. Lawrence River Area of Concern, and to compare lampreys and mussels as biomonitors of metals in tributaries to the St. Lawrence River were completed. Research on the biodiversity of freshwater mussels in the lower Great Lakes drainage basin continued. Highlights of two major studies are summarized below:

(1) Comparison of caged and native mussels (*Elliptio complanata*) as biomonitors of organic contaminants in the St. Lawrence River.

In 1990, Environment Canada, Ontario Region established a freshwater mussel biomonitoring program in the St. Lawrence River Area of Concern (AOC) to provide information on long-term contaminant trends, transboundary pollution, and the response of the system to remedial action. The program used the dominant native mussel species, *Elliptio complanata*. The program was terminated in 1996 due to the decimation of local populations of native mussels by the exotic zebra mussel (*Dreissena polymorpha*). In order to maintain continuity of the monitoring program and to salvage pre-remediation data, caged mussels of the same species were evaluated as alternative biomonitors. Mussels obtained from a reportedly clean lake (Balsam Lake, Ontario) were caged for 12 weeks at three sites in the AOC. Upon retrieval of cages, native mussels of the same species were also collected. Fifteen caged and 15 native mussels from each site, plus 15 pre-exposure animals, were individually analyzed for residues of organochlorine pesticides and PCBs. The data were analyzed to determine if caged mussels reflected concentrations in native mussels after the exposure period, if residues in caged mussels differed in terms of variability from those in native mussels, and if caged mussels showed the same site-to-site trends in contamination as native mussels.

Mussels from Balsam Lake were found to be more contaminated with aldrin, BHC, endosulfan and chlordane than native mussels from the St. Lawrence River. In all cases, caged mussels depurated these compounds to some extent, but generally did not quite reach the levels in native mussels. Balsam Lake mussels were less contaminated with Mirex, DDT and PCBs than those in the St. Lawrence River. Concentrations of Mirex in caged mussels were intermediate between those in pre-exposure and native mussels, whereas concentrations of PCBs in caged mussels reached levels as high as those in native mussels at all but the most contaminated site. In contrast, caged mussels did not accumulate DDT above background levels after 12 weeks exposure. Concentrations of chlorobenzenes did not vary significantly among Balsam Lake, caged or native mussels. In general, variability among individuals increased with increasing contamination. Despite this trend, concentrations in caged mussels were exceptionally variable for some

compounds. Variability of residues in caged mussels was more often similar to those in pre-exposure than native mussels for organic contaminants, whereas variability of metal residues in caged mussels had been shown in a previous study to be more similar to those in native mussels. Caged mussels usually ranked the study sites in the same order as native mussels in terms of the degree of contamination. However, they did not necessarily reflect the magnitude of the differences among sites. Caged mussels consistently accumulated fewer PCB congeners than native mussels at all sites. As a result, number of congeners in native mussels differed significantly among the three sites, whereas numbers of congeners in caged mussels did not. The results of this study suggest that caged mussels are less sensitive indicators of contaminant trends than native mussels, and that direct comparisons of data between caged and native mussels will be difficult. This research was partly funded by Environment Canada, Ontario Region.

(2) Assessment of the current conservation status of rare species of freshwater mussels in southern Ontario.

Nearly half of the 40 species of freshwater mussels native to the Canadian waters of the lower Great Lakes drainage basin are presently ranked as very rare (S2), extremely rare (S1) or known from historical records only (SH) by the Natural Heritage Information Centre in Peterborough, Ontario. In this study, we determined the current conservation status of 21 species of mussels believed to be the most at risk. Most of these species have severely declined in the Great Lakes themselves due to the impact of the zebra mussel (*Dreissena polymorpha*). As a result, the last refugia for many of Canada's native mussel species are the Grand, Thames and Sydenham Rivers in southwestern Ontario. Thirty-seven sites on these rivers that historically supported the target species were intensively surveyed during the summer of 1997 to determine the true status of these species. Current data on species distributions from this and other recent (post-1990) surveys on these rivers were combined and compared with the historical data to determine if there have been changes over time. The major findings of this study are as follows:

We successfully located more living species on all three rivers than other recent surveyors, due to our greater sampling effort. However, we still observed species losses: 27%, 41% and 24% of the species known from the Grand, Thames and Sydenham Rivers, respectively, according to historical records dating back to the late 1800s, were not found alive in 1997. Although 30 of the 36 species previously known from the study area were found alive, 13 of these species now occur in fewer rivers than they did in the past, i.e., their ranges have been reduced. The Sydenham River still supports the richest and most productive mussel community of any small river in Canada, with 25 living species, an average diversity of 13 species/site, and an average abundance of over 150 individual mussels/site based on a sampling effort of 4.5 person-hours. In 1992, Arthur H. Clarke urged "...that the Sydenham River be made an ecological preserve and that its fauna be protected by legislation." To this, we would add that time is of the essence.

The conservation status of 21 species of freshwater mussels was assessed by comparing the current (post-1990) distribution of each species with its historical distribution. On the basis of these comparisons, changes to the official conservation status ranks (Ontario's SRANKS) of 11 species were proposed. Three species currently ranked SH were found alive and could therefore be downlisted to S1 (*Epioblasma torulosa rangiana*, *Obliquaria reflexa* and *Toxolasma parvus*). As the persistence of *Simpsonaias ambigua* is highly likely due to the presence of fresh shells at

many sites, this species was also tentatively downlisted to S1. Five species appear to have declined significantly (*Fusconaia flava*, *Ligumia nasuta*, *Pleurobema coccineum*, *Truncilla donaciformis* and *Villosa iris*) and are therefore recommended for uplisting. Two other species (*Cyclonaias tuberculata* and *Truncilla truncata*) were more common than expected, and could be downlisted. We recommended that the 11 species with current or proposed ranks of S1 be given first consideration for national status designation by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC), as measures will have to be taken soon to prevent their extirpation. These species are: *Epioblasma torulosa rangiana*, *Obliquaria reflexa*, *Simpsonaias ambigua*, *Toxolasma parvus*, *Lampsilis fasciola*, *Obovaria subrotunda*, *Ptychobranthus fasciolaris*, *Utterbackia imbecillis*, *Villosa fabalis*, *Truncilla donaciformis* and *Pleurobema coccineum*. The study leader has been commissioned by COSEWIC and COSSARO (Committee on the Status of Species at Risk in Ontario) to prepare national and provincial status reports on three of these species (*Epioblasma torulosa rangiana*, *Lampsilis fasciola* and *Villosa fabalis*). During this study, a conservation status score system was devised for identifying and prioritizing areas of prime mussel habitat that should be protected. The system may be a useful tool for agencies that are responsible for managing the water and habitat quality of Ontario's rivers. This research was partly funded by the Endangered Species Recovery Fund.

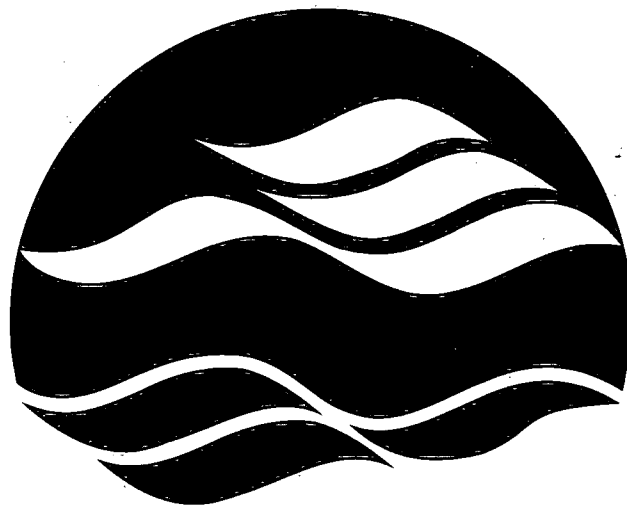
Articles/reports/conference presentations in FY 97-98

- (1) Metcalfe-Smith, J.L., S.K. Staton, G.L. Mackie and E.L. West. 1998. Assessment of the current conservation status of rare species of freshwater mussels in southern Ontario. Final report to the World Wildlife Fund Canada.
- (2) Metcalfe-Smith, J.L., S.K. Staton, G.L. Mackie and N.M. Lane. Changes in the biodiversity of freshwater mussels in the Canadian waters of the lower Great Lakes drainage basin over the past century. *J. Great Lakes Res.* (submitted)
- (3) Metcalfe-Smith, J.L., S.K. Staton, G.L. Mackie and N.M. Lane. 1998. Selection of candidate species of freshwater mussels (Bivalvia: Unionidae) to be considered for national status designation by COSEWIC. *The Canadian Field-Naturalist*, in press
- (4) Metcalfe-Smith, J.L., J. Biberhofer and M. Villella. A comparison of caged and native mussels (*Elliptio complanata*) as biomonitors of organic contaminants in the St. Lawrence River. Presented at the 24th Annual Aquatic Toxicity Workshop, Niagara Falls, ON October 20-22, 1997.
- (5) Renaud, C.B., H.K.T. Wong and J.L. Metcalfe-Smith. Trace metal levels in benthic biota from four tributaries to the St. Lawrence River, Quebec, Canada. Presented at the Fourth International Conference on Trace Metals in the Aquatic Environment, Kuala Lumpur, Malaysia, May 19-23, 1997.

ENVIRONMENT CANADA LIBRARY, BURLINGTON



3 9055 1016 4193 3



**NATIONAL WATER
RESEARCH INSTITUTE**

**INSTITUT NATIONAL DE
RECHERCHE SUR LES EAUX**

**National Water Research Institute
Environment Canada
Canada Centre for Inland Waters
P.O. Box 5050
867 Lakeshore Road
Burlington, Ontario
Canada L7R 4A6**

**National Hydrology Research Centre
11 Innovation Boulevard
Saskatoon, Saskatchewan
Canada S7N 3H5**

**Institut national de recherche sur les eaux
Environnement Canada
Centre canadien des eaux intérieures
Case postale 5050
867, chemin Lakeshore
Burlington; (Ontario)
Canada L7R 4A6**

**Centre national de recherche en hydrologie
11, boulevard Innovation
Saskatoon; (Saskatchewan)
Canada S7N 3H5**



Environment
Canada

Environnement
Canada

Canada