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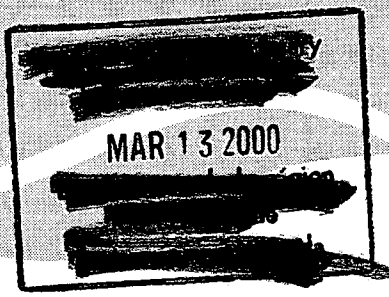
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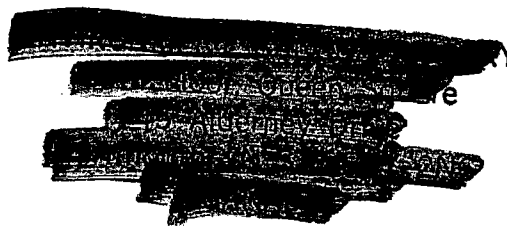
ANNUAL REPORT - FISCAL YEAR 1998-1999

Aquatic Ecosystem Protection Branch

NWRI Contribution No. 99-085

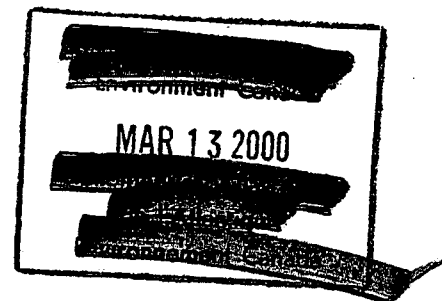
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Annual Report – Fiscal Year 1998-1999



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

NWRI Contribution No. 99-085



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Director's Introduction

This report presents an overview of the research carried out in fiscal year 1998-1999 in the Aquatic Ecosystem Protection Branch of Environment Canada's National Water Research Institute. We hope that this document will provide useful information to other government agencies, to universities and the private sector, to non-governmental organizations, to our Canadian and international collaborators, and to others. This report is also available electronically by contacting jenn.dykeman@cciw.ca.

R.J. Maguire
Director, Aquatic Ecosystem Protection Branch
National Water Research Institute

Mot du directeur

Ce rapport présente un aperçu des recherches effectuées au cours de l'exercice 1998-1999 à la Direction de la protection des écosystèmes aquatiques de l'Institut national de recherche sur les eaux d'Environnement Canada. Nous espérons que ces informations seront utiles aux autres organismes gouvernementaux, aux universités et au secteur privé, aux organisations non gouvernementales, à nos collaborateurs du Canada et d'ailleurs, ainsi qu'à d'autres. On peut aussi obtenir des exemplaires électroniques de ce rapport en communiquant avec jenn.dykeman@cciw.ca.

R.J. Maguire, directeur,
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The National Water Research Institute will be publishing, in both French and English, an annual report which will cover many of the topics in this report. For more information, or to obtain a copy, please contact:

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L'Institut national de recherche sur les eaux publiera, en français et en anglais, un rapport annuel qui inclura plusieurs des sujets du présent rapport. Pour de plus amples renseignements, ou pour en obtenir copie, veuillez vous adresser à:

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NWRI and AEPB Missions

The mission of the National Water Research Institute is to create and disseminate new knowledge and understanding of aquatic ecosystems for the resolution of environmental issues of regional, national or international significance to Canada.

The mission of the Aquatic Ecosystem Protection Branch is to conduct research to protect aquatic ecosystems from the deleterious effects of toxic chemicals by developing knowledge and understanding of priority pollutants to support informed decision-making and sustainable practices. AEPB research is directed towards a fundamental understanding of such issues as priority chemicals, regional ecosystems, pollution prevention, ecosystem sustainability and biodiversity.

AEPB is organized into three Projects, the Exposure and Effects of Priority Substances Project, the Non-Point Sources of Pollution Project and the Sources and Fate of Toxic Substances Project. This report describes the research carried out in each Project in fiscal year 1998-1999.

Current Research Summaries Fiscal Year 1998-1999

Exposure and Effects of Priority Substances Project

The Project provides important scientific information on the effects of toxic chemicals in Canadian aquatic ecosystems in support of Departmental hazard and risk assessments, and risk management activities. This knowledge is used by Environment Canada to make decisions under the Toxic Substances Management Policy, the Canadian Environmental Protection Act, the Environmental Effects Monitoring program, and major regional ecosystems programs, and to make decisions with regard to emerging issues such as endocrine-disrupting substances (EDSs). The Project accomplishes its goals by developing and applying techniques to screen and assess priority substances and effluents for their potential to cause impacts on the survival, growth, development and reproduction of aquatic biota. Assessing the impacts of priority substances and potential EDSs such as alkylphenols, natural and synthetic estrogens, municipal effluents, agricultural and urban runoff is a current focus of the Project. Collaborative research is done to assess the occurrence, persistence and fate of these and other toxic chemicals. The relative roles of toxic chemicals and environmental factors in affecting the biodiversity of aquatic ecosystems is also a Project focus.

Substances d'intérêt prioritaire: exposition et effets

Ce projet permet d'obtenir d'importants renseignements scientifiques sur les effets des composés chimiques toxiques dans les écosystèmes aquatiques, contribuant aux activités d'évaluation et de gestion des risques environnementaux du ministère. Environnement Canada applique ces connaissances dans ses processus de prise de décision aux termes de la Politique de gestion des substances toxiques, de la *Loi canadienne sur la protection de l'environnement*, du Programme de surveillance des effets environnementaux et des programmes sur les écosystèmes régionaux importants, ainsi que les décisions sur les nouveaux domaines comme celui des substances perturbatrices des fonctions endocriniennes (SPFE). Le Projet arrive à ses fins en développant et en appliquant des techniques pour identifier et évaluer les substances prioritaires et les effluents quant à leur potentiel à avoir des impacts sur la survie, la croissance, le développement et la reproduction des organismes aquatiques. Le projet met présentement l'accent sur l'évaluation des impacts des substances prioritaires et des SPFE potentielles comme les alkylphénols, les estrogènes naturels et synthétiques, les effluents municipaux et les eaux de ruissellement urbaines et agricoles. On étudie aussi les contributions relatives des produits toxiques et d'autres facteurs environnementaux sur la biodiversité dans les écosystèmes. La recherche concertée permet d'évaluer l'incidence, la persistance et le destin des produits réputés toxiques ou perturbateurs.

STUDY TITLE: *Environmental chemistry and effects of priority substances*

STUDY LEADER: M.R. Servos

STUDY TEAM: A. Jurkovic, R. McInnis

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 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 effluents, textile effluents, NPEs, 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 substances (EDS) in municipal effluents

Alkylphenol polyethoxylates, their metabolites, and natural and synthetic estrogens, have been identified as trace contaminants in municipal effluents around the world. These substances are suspected of causing a variety of estrogenic responses in fish, including induction of vitellogenin and intersex. The prevalence and potential effects of EDSs in Canadian municipal effluents was previously not well documented. A study of municipal effluent in Canada was initiated in collaboration with Wastewater Technology International, the Burlington Environmental Technology Office, local municipalities and other scientists at NWRI, with the following objectives: to determine the presence of alkylphenol polyethoxylates (including their metabolites), natural and synthetic estrogens in Canadian municipal effluents; to determine if exposure to municipal effluent causes estrogenic responses in fish; and to identify the substances responsible for estrogenic responses in Canadian effluents. Alkylphenols and their polyethoxylates are currently being evaluated under the Canadian Environment Protection Act (PSL2) and these data were required to support their environmental assessment. A literature search on the effects of alkylphenol ethoxylates and their degradation products was conducted as part of the Supporting Document for the PSL assessment. Alkylphenols were determined to be common contaminants in Canadian municipal effluents with significant amounts being released in final effluents (low $\mu\text{g/L}$) and sludges (low $\mu\text{g/g}$). Most of the release in final effluents was as nonylphenol polyethoxycarboxylates, which are potentially estrogenic chemicals or can be metabolized to nonylphenol. Natural and synthetic estrogens were also detected in final effluents of most Canadian sewage treatment plants at low ng/L quantities. Municipal treatment systems were found to be very effective at reducing the levels of both 17β -estradiol and estrone in final effluents. Toxicity Identification Evaluation (TIE) separations, using the estrogen receptor binding as the endpoint (YES), suggested that estrogenic responses are associated with the fractions containing natural and synthetic estrogens rather than alkylphenols. Although the natural estrogens are found at much lower concentrations than alkylphenols, they have much higher affinity for the estrogen receptor, and may therefore contribute to the estrogenic responses observed in the effluents. The concentrations of both alkylphenols and natural estrogens found in Canadian effluents are in the ranges reported in the literature for the threshold for estrogenic responses in fish. Exposures of immature trout for 18-21 d to the effluents in the streams or in the laboratory were conducted to determine the potential for estrogenic responses, including induction of plasma vitellogenin. These studies have

~~demonstrated the potential for estrogenic responses in fish exposed to Canadian municipal effluents although the response does not appear to be as dramatic as those reported elsewhere.~~

(2) Potential exposure of the environment to endocrine disruptors in runoff from intensive agriculture.

Applying manure to agricultural fields is a traditional method of utilizing and disposing of these organic wastes. Leachate from the manure enter adjacent streams resulting in eutrophication or even acute toxicity. Hog manure can contain high concentrations of endocrine-disrupting substances (EDSs), including natural estrogens, which can potentially disrupt normal endocrine (hormonal) and reproductive function in biota if exposure is sufficient. In response to these concerns a new collaboration between researchers in the National Water Research Institute (NWRI) and Agriculture and Agri-Food Canada was initiated. The goals include determining the persistence of EDSs in soil after manure application and establishing the identity, exposure and effects of EDSs entering adjacent streams following application. Studies confirm that high concentrations of EDSs are found in hog manure and these compounds have the potential to enter adjacent streams immediately after field application. Studies are underway to determine the effect of exposure of biota to estrogenic compounds as a result of intensive agriculture practices including the application of a variety of animal wastes.

(3) Exposure and effects of trace contaminants in lampricide (TFM) formulations

Toxicity identification evaluations of TFM formulations were conducted to isolate and identify the chemicals responsible for both MFO induction and estrogenic responses. It was determined that the MFO induction was caused by a unique dioxin-like compound while the estrogenic response as measured by binding to the estrogen receptor was caused by TFM itself. Studies of the temporal and spatial distribution of biological responses during regular lampricide treatments demonstrated that the MFO response is eliminated in less than 3 weeks. Fish exposed in cages did not show vitellogenin induction (an indication of exposure to estrogens).

(4) Using stable isotopes to understand complex food webs

Studies on Lake Erie to understand the energy flow and bioaccumulation of contaminants in food webs were completed. The seasonal dynamics of the ecosystem, especially the lower end of the food chain, was shown to be critical in controlling the isotopic signatures in higher organisms. This confounds the interpretation of results of simple correlations between isotopic signature and bioaccumulation of toxic chemicals unless considerable knowledge of the system is known. This work continues to contribute to our understanding of the structure and function of large lakes and increases our ability to predict energy transfer and the bioaccumulation of toxic chemicals in complex food webs.

STUDY TITLE: *Reproductive and Developmental Toxicology- Endocrinology*

STUDY LEADER: Scott Brown

STUDY TEAM: M. Vilella, M. Brown, N. Lane (contractor)

Overview:

This Study investigates the toxicological mechanisms of deleterious substances on fish growth, reproduction and development. It also develops new techniques to identify and monitor endocrine modulating capability, responses and effects of priority substances and effluents in aquatic biota in support of national priorities under the Clean Environment Business Line (e.g., TSMP, CEPA and Great Lakes initiatives [Great Lakes 2000 & COA Targets 2.1 for tier 1 and tier 2 substances]). Additionally, the Study produces new knowledge in support of national priorities under the Nature Business Line (e.g., the rehabilitation of degraded populations of native species [GL2000, COA Target 1.3.3] and protecting the function and structure of diverse, self-sustaining biological communities [GL2000, COA Target 3.31]). The aims of the project are: a) to understand the progression of toxicity of high-priority pollutants from the molecular level to effects at the whole fish level, b) to identify biochemical, endocrine and physiological responses in exposures to contaminants which will prove useful in studies on wild fishes. Changes in reproductive, developmental, hormonal or biochemical function often precede, but could also result from disturbed physiological processes. It is thus conceivable that toxic chemicals will disrupt these systems early in the development of toxic syndromes. Therefore, it is anticipated that early and sensitive indicators of toxicity may be obtained, along with insight of toxic mechanisms, by studying the possible interference of toxic pollutants in these systems. After responses are validated, techniques and knowledge are applied to natural fish populations.

Research:

(1) Exposure to Priority Substances and Effluents

i) The Effects of Endocrine Disrupters on Sea Water Adaptability, Growth and Survival of Salmon Smolts

Evidence from our preliminary findings indicates that environmental concentrations of both nonylphenol and estrogen may compromise successful parr-smolt transformation, which is critical to long-term growth and survival of salmon at sea. This represents a novel line of research examining the effects of endocrine disruption in the field and laboratory. The results will contribute to understanding of factors which may affect sustainability and management of Canadian and international stocks of Atlantic and Pacific salmon. The work will also be relevant for risk management of alkylphenols under the current CEPA PSL II assessment.

ii) Role of Organochlorines in Mechanisms Controlling Growth and Development

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. 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. Reviews of methodology to test thyroid status in contaminant exposed fish were completed. Impaired thyroid function were described in fish exposed to co-planar PCBs via their diet and antioxidant deficiencies were investigated in fish exposed to Hamilton Harbor effluents. For studies about the potential effects of hydroxylated PCBs on thyroid function tests in aquatic mammals and birds (Norstrom & Sandau, CWS), analyses have been completed and negative correlations between indices of thyroid and thyroid status and body contaminant burden noted.

iii) Impact of Natural and Synthetic Estrogens in Sewage and Agricultural Waste

The environmental persistence of estrogenic substances in domestic sewage and agricultural run-off (Servos, Burnison et al.) was investigated. Analyses of samples were completed, and improvements to extraction procedures to eliminate DOC interference with immunoassay procedures were described.

(2) 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 concerns regarding stocks of Baltic Sea salmon and trout. The first year of a collaborative project (Brown, EC; Fitzsimons, DFO; Honeyfield and Tillitt USGS-BRD; Michigan and Wisconsin DNRs; Wright GLFC) examining micronutrient dynamics in prey species from the lower Great Lakes is about 75% complete (June year-end). A joint project investigating the interactions among contaminants, thiamine deficiency and antioxidant vitamins (Fitzsimons and Sergeant, DFO) has been completed; data have been incorporated into a draft manuscript.

STUDY TITLE: Xenoestrogenic Screening Procedure to Identify Substances of Concern

STUDY LEADER: B.K. Burnison

STUDY TEAM: D.J. Nuttley, T. Neheli (contractor)

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 emphasised in most studies. Some of the chemical classes which have been shown to elicit these effects include alkylphenol ethoxylate surfactants (i.e., nonylphenol ethoxylate), octylphenol, bisphenol A, organochlorine pesticides, PCBs, dioxins, PAHs and phthalates in several industrial effluents, municipal sewage, and non-point sources. These estrogen disrupting chemicals (xenoestrogens) mimic the natural estrogen 17 β -estradiol (E₂) 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). We have successfully used the YES assay to determine the estrogenicity in selected sewage treatment plant effluents from across Canada. The bioassay was used as the biological endpoint in chemical fractionation procedures to determine specific causative compounds. The YES assay has also proven useful in determining the estrogenicity of hog farm waste which is sprayed on agricultural lands.

(1) Municipal sewage effluents

During the course of the year three separate effluents have been fractionated including the Galt (Cambridge), Adelaide (London), and Toronto Main STPs. Effluent was filtered and one portion acidified to pH 3.0. Both acidified and unacidified effluents were individually passed through ENVI C-18 disks and washed with 25% methanol. The disks were eluted with methanol and an aliquot of the methanol eluate was chemically fractionated on a reverse-phase C-18 HPLC column. The YES bioassay was run on each one mL fraction. The majority of the activity correlated to compounds eluting at the estradiol retention time, then at ethinylestradiol, followed by estrone. Estrogenic activity was also present in the hydrophobic fraction near the retention times of octylphenol and nonylphenol. There was also some activity at the retention time of estradiol acetate. The acidified samples seem to give a higher activity in the estrone retention time area than the unacidified. These HPLC fractions were also tested chemically for estrogens and alkylphenols. For Toronto Main STP there was 0.04 μ g/L 4-t-octylphenol and 0.32 μ g/L 4-nonylphenol. The GC/MS chemical analyses of the estrogens yielded 7.1 ng/L estradiol, 7.7 ng/L ethinyl estradiol, and 13.1 ng/L estrone. The HPLC procedure we have developed can also detect testosterone, between estradiol and ethinyl estradiol. We have also been part of a survey across Canada looking at the estrogenic activity using the YES assay in various sewage treatment plants. The results of the YES bioassay are only semi-quantitative. Currently, values are reported as a percentage of the response given by a one nanomolar concentration of 17 β -estradiol.

(2) Hog Waste

Some farmers in southern Ontario spray their fields with hog waste during late April. We analysed the estrogenic activity in the original hog waste from three farms, the drainage tiles from the fields, and upstream and downstream samples. High estrogenic activity was noted in the original hog waste, especially from Site 1, which was expected since this site contains 700 sows. Although the YES bioassay is not intended to be a quantitative assay, we estimated the activity in the hog waste by comparing the response to estradiol. We found approximately 7.2 µg estradiol equivalents per litre. This compares to 4.9 µg/L E₂-Eq. for the EIA procedure and 0.58 µg estradiol (analysed chemically). This result is not unusual, since the YES bioassay is not as specific as the EIA or estradiol determinations. Hydrophobic compounds in the hog waste were concentrated by C₁₈ SPE and analysed by HPLC. A polar peak exhibiting YES estrogenicity was noted and subsequently isolated and sent to Germany (Dr. T. Temes, Wiesbaden) for identification using MS/MS. During routine monitoring of estrogenic activity in the receiving water upstream of the field drainage tiles, a very high activity above the Site 1 hog farm was observed. The source of this activity remains unknown.

The potential contribution of estrogen conjugates (glucuronides and sulphates) to the YES bioassay results and the fate of these conjugates in the sewage effluent are of concern. We are currently investigating the use of the specific enzymes (glucuronidase and sulfatase), as well as acid hydrolysis, to detect the presence of these conjugates.

(3) Dissolved Organic Matter and Cadmium

Two German scientists, Drs. Thomas Meinelt and Michael Pietrock, spent the month of June working in our laboratory under the auspices of the Canada/Germany Bilateral Agreement on Ecosystem Health. We investigated the role of dissolved organic matter (DOM) on the uptake of cadmium into zebrafish embryos. Preliminary results show that the chorion ("egg shell") provides excellent protection for the developing embryo. DOM, depending on the source, does combine with cadmium and lowers the amount of cadmium on the chorion. In addition, acute cadmium toxicity tests were performed in the presence and absence of DOM.

STUDY TITLE: Development and application of quantitative structure-activity relationships to acute, chronic, reproductive and developmental data

STUDY LEADER: K.L.E. Kaiser

STUDY TEAM: V. Palabrica, S. Niculescu (contractor)

Research progressed on the development and application of neural network methodology for the prediction of toxicity values. Specifically, predictability of fathead minnow acute toxicity values increased and values for approximately 750 substances were predicted. This work applies a probabilistic neural network (PNN) methodology as developed and refined under contract with Dr. Stefan Niculescu. This work was supported by a grant from the Commercial Chemicals Evaluation Branch (CCEB), who also specified the 750 substances for which the toxicity was predicted. A description of the methodology and all values predicted are explained in a Technical Note (Kaiser, K.L.E. and S.P.Niculescu, AEP-TN99-001).

There are over 20,000 substances on the Domestic Substances List, most of which are individual substances with clear identifiable structures. Existing computer modeling programs, such as Ecosar and Topkat, cannot provide any answer for many of these substances, and for those which are within their capability, degrees of confidence on the data is not available. In order to compare these methodologies with the Probabilistic Neural Network (PNN) methodology developed here, CCEB commissioned an independent contractor to evaluate the results. Based on 63 substances for which all three methodologies were able to provide estimates (PNN data are available for all substances), the PNN was found to be superior. For example, the average square error was only 0.36, versus 0.91 for Ecosar and 1.48 for Topkat for these test substances. Furthermore, the other methods give estimates up to 6 orders of magnitude from measured values, and have a number of values in excess of 2 orders of magnitude from measured values. In contrast, the PNN estimates do not have a single value in excess of 2 orders of magnitude and much fewer numbers in excess of one order of magnitude, when compared with Ecosar and Topkat estimates. A Note of Caution was also published in collaboration with several colleagues from the USA, England, and Germany.

Work in the laboratory continued with the analysis of biological, sediment, and sludge samples, including those from collaborative studies with AAFC researchers on endocrine disrupting materials in manure and contaminants in biological samples from the Great Lakes basin and northern rivers areas.

STUDY TITLE: Fate and Effects of Contaminants on the Aquatic Ecosystems

STUDY LEADER: T. Mayer

STUDY TEAM: M. Servoš, J. Marsalek, M. Brown, A. Jurkovic, R. McInnis,
Q. Rochfort (contractor),

Research on the fate and effect of priority substances, entering the receiving waters from various sources continued focusing on the investigation of the toxicity of highway runoff and on the impacts of road salts on the small urban water bodies. An exploratory investigation was carried out to assess the potential contamination of surface waters from animal waste applications. The major activities are summarized below:

(1) Toxicity of highway runoff

Highway runoff has been identified as an important contributor of non-point source pollution. Highway discharges contain a broad variety of contaminants, including road salts, heavy metals and polycyclic aromatic hydrocarbons (PAHs). Because of their potential toxicity, many of these compounds have been nominated for consideration under Track 1 and 2 of TSMP and Priority Substances List (PSL) 1 and 2 of CEPA. The aim of the study was to identify and quantify the toxic substances in highway runoff and establish linkages between the contaminant concentrations in runoff and associated toxicity. Specifically, the effect of traffic volume and composition as well as temporal effects on the contaminant levels and runoff toxicity were investigated. A battery of bioassays was used to assess the degree of runoff toxicity.

Variation in toxic responses was observed both seasonally and within the runoff event. Runoff samples containing high concentrations of road salts from winter maintenance were acutely toxic to *Daphnia magna*. The results of the sub-mitochondrial (SMP) tests were consistent with the *D. magna* tests. Elevated concentrations of zinc from the bridge infrastructure were responsible for positive toxic responses from the site with lowest traffic density. Within a single event, a sharp decline was observed in runoff toxicity to *Ceriodaphnia dubia*, confirming the high toxicity of the "First flush". Significant Mixed Function Oxidase (MFO) induction to rainbow trout was observed, which was consistent with the high concentrations of PAHs in road sediments. Runoff from the multilane highway, with the highest traffic density, induced the strongest MFO response.

(2) Effect of road salts on the benthic compartments of aquatic systems

Although some information exists on the adverse effects of road salts on aquatic organisms, little is known about the effects of road salts on the benthic environment, particularly on porewater chemistry. Considering that the gradual buildup of a saline layer near the bottom of lakes would most impact the benthic environment, information is needed on the effect of salts on the benthic compartment of lentic systems. Sediment porewater chemistry is a particularly important part of this assessment. A comprehensive study was carried out to investigate the impacts of road salts on the benthic compartment of a small urban detention facility, Rouge River Pond. Although the pond is an engineered water body designed primarily for hydraulic flow management and stormwater treatment, it is representative of many small urban lakes, ponds and wetlands which receive road runoff and are probable high impact areas. Specific objectives of the study were to document the porewater chemistry of an aquatic system affected by elevated salt concentrations and to carry out the toxicological assessment of sediment porewater to determine what factors may cause porewater toxicity. To meet the objectives porewater chemistry was determined and a geochemical model was

formulated to assess the salt impact on metal speciation. Solid phase metal chemistry was also assessed using Tessier's protocol. The benthic invertebrate *Hyalella azteca* was used as a test organism in the toxicity assessment. The results indicate that the sediment porewater, which is in a steady state with the overlying water, enriched in inorganic salts, may itself attain high salt concentrations. The computations show that increased chloride levels have important implications on the Cd complexation, augmenting its concentration in porewater. The toxicity tests suggest that the toxicity in porewater is caused by metals rather than high levels of chloride or $\text{NH}_4\text{-N}$.

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

STUDY TEAM: I.M. Scott, S.K. Staton (contractor), G.L. Mackie (University of Guelph), D.J. Berg (Miami University)

Studies to determine the range, population stability and environmental requirements of rare species of freshwater mussels in southern Ontario continued. A related study to examine the influence of sampling effort on the detection of rare species was also conducted, and national and provincial status reports were prepared for three species of freshwater mussels at risk in Canada. A study to determine the degree of genetic similarity between populations of several Ohio State-listed species of mussels in the Ohio River drainage and populations from Lake Erie drainages in Ontario was initiated, and a study to examine the effects of endocrine-disrupting substances on freshwater mussels in the Grand River, Ontario was also begun. Highlights of three studies are summarized below:

(1) Range, population stability and environmental requirements of rare species of freshwater mussels in southern Ontario.

An earlier review of historical data from 1860 to 1996 on the distributions of native freshwater mussels throughout the lower Great Lakes drainage basin provided compelling evidence that the steady decline in mussel diversity that has been documented in the United States is also occurring in Canada. In 1997, 37 sites on the Grand, Thames and Sydenham Rivers in southwestern Ontario were surveyed to determine the current conservation status of 21 species believed to be the most at risk. In this study, 20 additional sites on these rivers, as well as 9 sites on two rivers in the lower Lake Huron drainage, were surveyed to determine the ranges, population stability and environmental requirements of nine species, including three species (*Epioblasma torulosa rangiana*, *Villosa fabalis* and *Lampsilis fasciola*) that we have recommended for national status designation by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC).

Continuous, reproducing populations of *E. t. rangiana* and *V. fabalis* were found in a 45-50 km stretch of the middle Sydenham River, and a low density population of *E. t. rangiana* was also discovered in the Ausable River. As *E. t. rangiana* is listed as federally endangered in the United States, with only one or two known reproducing populations left, the Sydenham River population may be globally significant. *Lampsilis fasciola* was found alive in the Grand, Thames, and Maitland Rivers, with the healthiest population occurring in a 60 km stretch of the middle Grand River. Its continued existence may be threatened by over-exploitation of its fish host, the smallmouth bass. Significant findings for other target species included the discovery of two species in the Sydenham River that were previously thought to be extirpated from Ontario (*Epioblasma triquetra* and *Simpsonaias ambigua*), and the complete absence of live *Obovaria subrotunda* from the study area - indicating that it has declined alarmingly and may now be extirpated. New information on these and three other species, *Obliquaria reflexa*, *Ptychobranchus fasciolaris* and *Pleurobema coccineum*, may justify formal assessments of their national conservation status. Fish hosts for many of these species in Canada are unknown, but the distribution patterns for mussels and fishes in the studied rivers implicate darters (*Etheostoma* and *Percina* spp.) as potential hosts for several rare species of mussels. The Grand, Sydenham and Ausable Rivers were found to be significant refuges for one or more of the target mussel species. However, the Thames River has lost nearly one-third of its native

mussel fauna, and many species that remain do not appear to be reproducing. As the Sydenham River is now believed to support the most diverse mussel community of any Canadian or American tributary to the Great Lakes, the wise management of this system is critical to the preservation of the native mussel fauna of the region.

Results of this research warranted the preparation of provincial (COSSARO) and national (COSEWIC) status reports on three species of mussels believed to be among the most at risk in Ontario and Canada. The status of "Endangered" was proposed for the Northern Riffleshell (*Epioblasma torulosa rangiana*) and the Rayed Bean (*Villosa fabalis*), and the status of "Threatened" was recommended for the Wavy-rayed Lampmussel (*Lampsilis fasciola*). These three species will be among the first four species of freshwater mussels to be officially listed in Canada; all four will be designated at the annual meeting of COSEWIC in April 1999.

(2) Effect of sampling effort on the detection of rare species, estimates of total diversity, and apparent composition of the mussel community.

Comparisons between mussel surveys conducted in 1997 and other less intensive surveys conducted at the same sites in recent years showed that a greater sampling effort resulted in the discovery of significantly more species, as well as individual mussels, at a given site. In 1998, the effect of sampling effort (i.e., search time in person-hours) on the detection of rare species, the total number of species found, and the apparent composition of the mussel community was determined quantitatively using data obtained after 1.5, 3.0 and 4.5 p-h of sampling effort at all 29 sites surveyed. Results showed that an increase in sampling effort dramatically improved the detection of rare species. Fewer than 50% of the species defined as "rare" (<1-5 specimens per site) were detected after 1.5 p-h of effort, which is the sampling effort most commonly used in mussel surveys. In fact, 70% of encounters with the three main target species, *Epioblasma torulosa rangiana*, *Villosa fabalis* and *Lampsilis fasciola*, would have been missed if the search had ended after 1.5 p-h. Results also showed that an increase in sampling effort may significantly alter the apparent community composition, and hence our perception of which species are dominant and which are minor components of the community. The fact that new species were still being found in the last time interval at 65% of the sites suggests that even a relatively intensive sampling effort of 4.5 p-h may not locate all species present at all sites. It is clear that a significant amount of information about the mussel community will be lost if sufficient sampling effort is not used.

(3) Induction of Vitellogenin (Vg)-type proteins in freshwater mussels exposed to sources of endocrine-disrupting substances in the Grand River, Ontario.

Bivalves have a poorly understood endocrine system relative to other invertebrates, but it is known that a form of vitellogenin (Vg), the protein that is the precursor of egg yolk in oviparous vertebrates, is present in clams and mussels. The alkali-labile phosphate (ALP) assay was recently adapted by scientists at the Centre Saint-Laurent for measuring the induction of Vg-type proteins in marine bivalves exposed to endocrine-disrupting substances (EDCs). A field study to determine if seasonal changes in Vg-type proteins occur naturally in freshwater mussels in response to their reproductive cycle, and if exposure to EDCs released from urban point sources or through agricultural activities cause induction of Vg-type proteins in these mussels as measured by the ALP method, was conducted in 1998. In this study, we observed seasonal changes in Vg-type proteins in the hemolymph of *Lasmigona costata* that correlated with their reproductive cycle. It also appeared that levels of Vg-type proteins were greater in *L. costata* living downstream than upstream of an STP outfall, and that this effect decreased with increasing distance from the source. As there was considerable variation in the data, further work to improve the resolution of the ALP method was

indicated. In particular, the reproductive stage of the mussel must be known more precisely through histological examination of the gonad tissue.

Two additional studies were conducted in 1998-99. In the first study, mussels (*L. costata* and *Lampsilis siliquoidea*) were caged above and below STP outfalls in both London and Stratford, Ontario for three weeks. The experiment ran concurrently with a fish exposure such that the results could be compared and validated against the standardized fish response. In the second study, mussels (*Elliptio complanata*) were exposed to 17β -estradiol in the laboratory for three weeks. Sampling of mussels in the control and treatment groups was conducted after 0, 48h, 96h, 7d, 14d and 21d of exposure. Samples from these two studies are currently being analyzed, and laboratory and field testing of the ALP assay will continue next fiscal year. Identification of the characteristics of Vg-type proteins in mussel hemolymph using gel-electrophoresis will also be pursued next year, as a step towards the development of a more sensitive, antibody-specific assay for the detection of Vg-type proteins in freshwater mussels.

Non-Point Sources of Pollution Project

The Project provides important scientific information on sources, transport, effects and control of non-point source pollution impacting Canadian aquatic ecosystems, and thereby supports the Clean Environment and Nature components of the Departmental Management Framework. This information is used by Environment Canada in the management and delivery of regional ecosystem programs, and also to make decisions under the Toxic Substances Management Policy and the Canadian Environmental Protection Act. The Project accomplishes its goals by researching processes governing the generation, transport and prevention of non-point source pollution, and also by providing related services to others. Current activities include the assessment of wet-weather pollution from urban and agricultural lands; transport of pollutants from such sources; interactions of pollutants with sediment, flocs and biofilm; the modelling of pollution source/effect relationships; and mitigation of non-point source pollution by best management practices. All current studies include collaborative research with others.

Sources diffuses de pollution

Le Projet fournit d'importants renseignements sur les sources, le transport, les effets et les mesures de contrôle de la pollution diffuse, ayant un impact sur les écosystèmes aquatiques canadiens, contribuant aux composantes Environnement sain et Nature du cadre de gestion du ministère. Cette information est utilisée par Environnement Canada dans la gestion et la prestation de programmes ayant trait aux écosystèmes régionaux et dans la prise de décision dans le cadre de la Politique de gestion des substances toxiques et de la *Loi canadienne sur la protection de l'environnement*. Les objectifs du projet sont atteints par la recherche sur les mécanismes de production, de transport et de prévention de la pollution diffuse. En outre, l'équipe fournit des services connexes à d'autres intervenants. Parmi les activités principales on retrouve: l'évaluation des effets des eaux de ruissellement en milieux urbains et agricoles, le transport des polluants dans ces milieux, les interactions des polluants avec les sédiments, flocs et biofilms, la modélisation des rapports entre les sources et effets des polluants, et l'atténuation de la pollution diffuse par la modifications des pratiques agricoles ou de la gestion des eaux urbaines. Toutes les activités de recherches se font dans le cadre d'équipes pluridisciplinaires.

STUDY TITLE: Protection of Aquatic Ecosystems Against Deleterious Effects of Urban Non-Point Sources of Pollution

STUDY LEADER: J. Marsalek

STUDY TEAM: J. Heidt, B.G. Krishnappan, G. Larkin (contractor), Q. Rochfort (contractor)

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

Investigation of urban non-point pollution sources dealt with microbiological pollution, thermal pollution and the assessment of toxicity of stormwater and combined sewer overflows (CSOs). Urban stormwater and CSOs are recognized as significant sources of fecal bacteria, contributing to closure of swimming beaches when microbiological water quality guidelines, including the indicator organism limits (IOLs), are exceeded. Comparison of two Canadian guidelines, one based on a single rule (i.e. the IOL defined as a geometric mean) and the other based on two rules (i.e. the geometric mean and a maximum) indicated that when the number of samples is less than 15, the former is more conservative and vice versa. These findings are helpful for application of recreational water quality guidelines.

In warm weather, urban runoff collects heat from impervious surfaces (roofs, roads, parking lots) and thereby contributes to the thermal pollution of receiving waters. A methodology for predicting the thermal enhancement of stormwater runoff from paved surfaces was developed using a thermal energy balance and a one-dimensional heat equation for surface temperature of, and temperature gradient in, asphalt during wet and dry weather periods. Computer simulations of pavement temperatures compared well with measurements of temperature in the test plot. The model developed can be used to predict thermal loading in runoff from impervious areas.

(1) Toxicity of stormwater and combined sewer overflows

Potential toxic impacts of urban stormwater and CSOs were assessed by toxicity testing of various non-point sources. An exploratory study of urban runoff toxicity was undertaken at 14 urban sites, using a battery of five tests. About two fifths of all data did not show any toxic responses, one fifth indicated potential toxicity, one fifth confirmed toxicity, and one fifth severe toxicity. When comparing urban stormwater toxicity to that of runoff from a multi-lane divided highway (MLDH), the main difference was noted for the severe toxicity level; almost 20% of MLDH samples were severely toxic compared to just 1% of urban stormwater samples. Practically all severe toxicity detections were made during winter months, as a result of increased production of pollutants; their accumulation in, and quick release from, snowpacks; enhanced mobility of metals in chloride-laden snowmelt; and, high concentrations of de-icing salts in highway runoff. The last point was further confirmed in a study of toxicity of runoff with high levels of de-icing salts.

In CSOs, frequencies of toxicity detection were rather low (less than 10% of all samples) and, consequently, less severe (non-acute) forms of toxicity were addressed. In CSO samples possessing any form of toxicity, the Ames test produced positive responses in 44% of all cases, and chronic toxicity tests produced 35% of positive responses. Thus, with respect to CSO toxicity, genotoxicity and chronic toxicity are the primary concerns, rather than acute toxicity.

(2) Pollution Transport

Pollution transport was addressed in two studies. The first one dealt with simulation of urban runoff by a neural net. A cascade correlation neural net was found suitable for simulating runoff from rainfall data and predicting flows with a lead time of up to 20 minutes. The other study dealt with transport of suspended solids (and associated contaminants) in a stormwater pond. Seasonal surveys of suspended solids in the pond indicated that the observed suspended solids were mostly composed of flocs, with maximum sizes ranging from 30 to 212 μm for winter and summer surveys, respectively. It was further noted that naturally formed flocs, in the size range from 5 to 15 μm would settle faster than both smaller primary particles of higher density, and somewhat larger flocs of lower density, which are however susceptible to break up by turbulence

(3) BMPs for stormwater management

Impacts of stormwater discharges on receiving waters can be prevented or reduced by best management practices (BMPs). New information was produced on four BMPs: oil/grit separators, infiltration facilities, stormwater ponds and biofilters. A new catchbasin design, serving as a grit separator, was developed for a private sector client. Compared to the conventional design, this new catchbasin traps coarse floatables and retains 10% more of fine sand entering the catchbasin. The significance of these enhanced removals will be assessed with respect to long-term performance. The experience with stormwater infiltration facilities was reviewed. It was noted that well designed facilities can infiltrate relatively clean or pretreated runoff from small urban areas and produce such benefits as enhanced groundwater recharge and baseflows in small streams, and reduced runoff volumes, peaks and pollution loads. For larger areas or in cold climate, infiltration facilities should be combined with storage facilities for runoff detention. The main concerns in the design and implementation of infiltration facilities are their operating life and the prevention of contamination of groundwater and soils.

Stormwater ponds are effective in removing suspended solids and the associated contaminants from stormwater. Where the pond does not provide a sufficient level of treatment, the pond effluent can be polished by another process. Experience with pond effluent polishing by a biofilter was reported for a field installation operated over three field seasons. Substantial removal of suspended solids (97%) from the influent stormwater resulted in a significant accumulation of sediment in the biofilter, which interfered with the system's main treatment objective, removing soluble nutrients through bacterial assimilation. This sediment was marginally to significantly polluted by Cd, Cr, Cu, Fe, Ni and Zn. Removal efficiencies of total organic carbon and the suspended orthophosphate decreased with time, but good removal of ammonium nitrogen (64%) was sustained independently of time or sediment accumulation. Frequent backwashing would be required to maintain optimal biological filtration conditions.

(4) Bioleaching of Sediments

Stormwater ponds accumulate significant quantities of contaminated sediments, which have to be periodically removed and disposed. The cost of such operations could be reduced by decontamination of these sediments. Consequently, the feasibility of sediment bioleaching was investigated. In this process, specific strains of naturally occurring, acid-producing bacteria were used to reduce the pH of sediment slurry, thereby solubilizing adsorbed and particulate metals into the liquid phase, for collection and processing. For retention times as short as 1.3 days, almost

complete solubilization of copper and zinc was achieved, and chromium and lead were partially removed. The process was found feasible for remediation of contaminated pond sediments.

Finally, the issues of stormwater operation and maintenance were also addressed with respect to three stages: initial pond design, regular operation and maintenance, and retrofitting. Suggestions for a future course of action include regular revisitation of the facility, developing a better understanding of the catchment/pond system using the ecosystem approach, and education of all stakeholders.

STUDY TITLE: Aquatic Sediment Structure: Implications for non-point source sediment and contaminant transport.

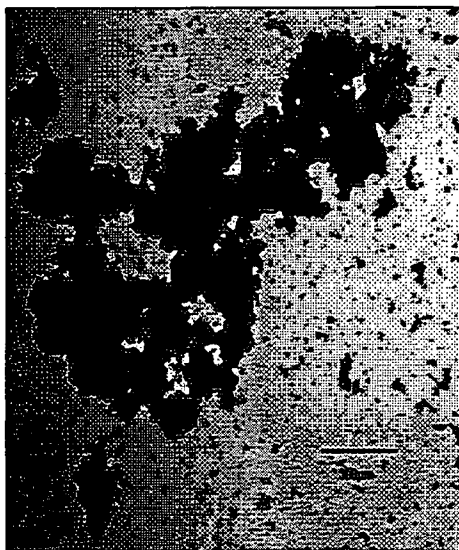
STUDY LEADER: I.G. Droppo

STUDY TEAM: C. Jaskot, B. Trapp

Research was continued in the area of floc architecture and its influence on floc function and behaviour, stormwater management of combined sewer overflows and stormwater runoff for the Kenilworth sewershed, and transport and erosion characteristics of contaminated bed sediment. 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 biological activity. Perhaps most importantly, toxic chemicals, including most heavy metals, the majority of priority pollutants and many other unlisted but environmentally sensitive chemicals, have environmental pathways that are primarily or exclusively associated with inorganic and bioorganic particles or flocs. There is, however, a fundamental lack of knowledge on the controlling factors of flocculation and how the actual structure of a floc influences its physical, chemical and 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 and their influence on the settling and transport of flocculated sediment will help to improve our knowledge of floc behaviour in natural and engineered systems.



Freshwater floc (16-Mile Creek, Oakville, Ontario).

(2) Transport/Erosional Characteristics of Contaminated Bed sediment.

Hamilton Harbour, Ontario, is one of 42 Areas of Concern (AOC) around the Great Lakes designated by the International Joint Commission (IJC) due to environmental impairments. As with the majority of AOCs, Hamilton Harbour has been identified as having impaired bed sediments due primarily to anthropogenic inputs of heavy metals and PAHs. Because there is busy ship traffic within Hamilton Harbour, concern has been raised over the possibility of the resuspension of this contaminated sediment by propeller wash, with eventual contamination of cleaner areas of the Harbour.

Experiments on the erosion characteristics of contaminated bed sediments from Hamilton Harbour as well as controlled flume experiments with kaolinite clay were conducted in an annular flume. It was discovered that the way in which the bed deposited (i.e. deposited under quiescent conditions, or under shear) had a strong influence on the stability of the bed. The amount of stress required to erode beds deposited under shear was up to eight times larger than for beds deposited under quiescent conditions. In addition, it was discovered that the presence of biofilm had very significant impacts on the erosion resistance of the bed as well as on the behaviour of the sediment flocs brought into suspension. The results of this study are relevant to any aquatic environment where sediment remobilization is of concern and should be considered when the transport of sediments and associated contaminants are being modelled.

(3) Combined Sewer Overflows and Stormwater Runoff

This study stems from the need to assess the current inputs and fate of contaminants to Hamilton Harbour (an IJC AOC) from a variety of sources in order to ensure that any proposed remediation will produce the desired results. This study focused specifically on the Kenilworth sewershed (a residential, commercial and heavy industry area) impacting Hamilton Harbour. The major sources of contaminants include: combined sewer overflows (CSO), coal pile runoff and direct industrial cooling water discharges (monitored by the MISA program of the MOEE). Multiple storm events were sampled for the CSO and coal pile runoff as well as a wide distribution of street sediments. All samples were analyzed for a host of metals and PAHs. Both the contaminant levels and loads from these sources were evaluated in assessing the potential impact they may have on the success of sediment and aquatic remediation in Hamilton Harbour. 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.

STUDY TITLE: Quantitative Risk Assessment

STUDY LEADER: A.H. El-Shaarawi

Research activities were focused on reviewing, developing and applying quantitative probabilistic methods for improving our understanding of risk assessment and risk management. Three case studies were the main motivation of the research: oil contamination from ships in Canadian Atlantic coastal waters; accumulation of contaminants in the tissue of fish in the Great Lakes; and microbiological standards and regulations for recreational and drinking waters. In addition, these case studies provided a frame work for technology transfer to our regional partners in CCIW and Atlantic Canada. An overview is presented below for each case study.

(1) Oil matching study

An oil sample can be characterized by its total ion mass spectrum, each ion in turn consisting of a number of peaks. Samples collected from the field don't display exactly the characteristic spectrum of the source oil because of weathering. As a result, the magnitude of the peaks are not the primary focus for source identification. Instead the profiles of the relative peaks are used as the focus of the analysis. An interactive multimedia application program has been developed for importing analytical results from the gas chromatograph to allow the user to compare "fragmentograms" of selected samples. It also reformats the data into a matrix form, suitable for performing classification and discrimination between the samples. Several data sets (some representing control samples) have been analysed to test the performance of the software and to evaluate its scope, particularly the dimension reduction methods.

(2) Contaminant level in fish tissue samples

Modelling the dependence of the accumulation of PCBs in fish tissue samples on growth parameters (age, length, weight and fat) have been completed using fish samples from 61 locations in Lake Superior and 177 locations in Lake Ontario. Further evaluation of the data confirmed the presence of strong dependence among the three growth parameters and allowed the development of a single growth index (the best linear combinations of age, length, weight and fat) which is used to model the PCB level in the tissue. The results identified three stages for the accumulation of PCB in the tissue: a) stage 1 shows a rapid decline of the concentration as the growth index increases to a minimum; b) stage 2 shows a switch in the pattern from decline to rapid accumulation of PCB in the samples; c) stage 3 shows that increase continues, but with a continuously reduced velocity which reaches its constant maximum value. The pattern is basically the same for the two lakes but the rate of change is faster for Lake Ontario than that of Lake Superior. The growth of contaminants is well described by a logistic model in which the maximum contaminant level for fish in Lake Ontario is shown to be three times that found in Lake Superior. In addition the maximum level is reached at a younger age for the Lake Ontario fish. The link between PCB loading to the lake and the PCB concentrations in tissues will be valuable in the formulation of regulation settings and in understanding the dynamics of fish exposure to PCB. In addition the developed methods can be used to model exposure to other contaminants.

(3) Microbiological Regulations

Regulations and guidelines do not provide complete protection to water users. Many factors are responsible for this, due to the source of water, the exposed individuals and the mode of exposure. Insight into these factors are gained by developing stochastic methods for assessing the performance of guidelines and regulations, which are discussed in two papers, where unavoidable sources of uncertainties in guidelines specifications and their field applications are described. Although the discussion is focused on two Canadian guidelines for microbiological recreational water quality, the issues considered are relevant to the wider scope of establishing and assessing the effectiveness of guidelines. Specifically, uncertainties in the selection of the indicator organism and its associated limits are discussed. The effectiveness of the limits, and the frequency of sampling is examined through model formulation and within a hypotheses testing framework. Simulation results show that the Canadian federal two-rules guideline is superior to the single rule provincial guideline when the number of monthly samples exceeds 15. The reverse is true for smaller sample sizes.

STUDY TITLE: Fine sediment transport and interactions with contaminants and biota.

STUDY LEADER: B.G. Krishnappan

STUDY TEAM: J. Marsalek, R. Stephens

Fine sediments play a major role in the transportation of hydrophobic contaminants in river systems and hence a knowledge of their transport characteristics is an essential prerequisite for modelling contaminant transport and interactions with biota. In this study, the transport characteristics of fine sediment under rough boundary conditions were studied in the rotating circular flume of the National Water Research Institute. In addition, transport characteristics of fine sediment from a variety of environments including a tile drain in an agricultural watershed and a storm water management pond in Kingston, Ontario were systematically studied in the laboratory. Some of the significant results of the current studies are outlined below:

(1) Measure and model flow characteristics and turbulent intensities in the rotating flume under rough boundary conditions and test the influence of boundary roughness on the transport characteristics of fine sediment.

The flow characteristics in rotating flumes under rough boundary conditions are not very well understood and there is very little work on this topic reported in the literature. Much of the reported work deals with smooth boundary flows. Since the transport processes of fine sediment including the flocculation process are governed by the flow turbulence, the roughness characteristics of the flow boundaries are likely to play a significant role in the transport of fine sediment. In this study, the influence of the bed roughness was systematically studied using the rotating flume. The roughness elements used in the study were river wash sand of median size 2mm. The sand was glued to a series of plastic plate-segments that fitted inside the flume and formed the bottom boundary. The flow fields were established by rotating the flume and the lid at different speeds and the bed-shear stress across the width of the flume was measured using a newly constructed Preston Tube assembly suitable for rough boundaries. The measured shear stress distributions were compared with those predicted using the PHOENICS model and a satisfactory agreement between the two were found. A further testing of the Preston Tube Assembly data will be carried out in the next study year by performing an independent measurement of bed-shear stress from the vertical distribution of the tangential velocities measured using the Laser Doppler Anemometer.

The Preston Tube assembly used in this study is a novel instrument and it is used for the first time to measure the bed shear stress in rough boundary flows. The adoption of the instrument for field use and its potential for commercialization will be explored in future studies.

(2) Model the transport of tile drain sediment in the receiving stream in the Kintore watershed (Collaboration with Dr. Mike Stone of the University of Waterloo).

The disposal of solid and liquid forms of livestock manure on agricultural land has created concern for its potential undesirable effects on surface and subsurface water quality. Liquid manure has been observed to flow through the soil column via large cracks and macropores to the groundwater or to tile drains where it is transported directly to receiving streams. Several studies have focused on the

impacts of manure spreading on water quality but little is known about its effects on the nature and transport characteristics of tile sediments in streams.

This controlled field study examines the effect of manure application and irrigation on the transport of tile sediment in an agricultural stream. Liquid manure was applied to a field-scale no-till corn plot (60 m x 72 m) on a tile drained silt loam soil located near Kintore in southern Ontario. Approximately 24 hours after manure application, the field plot was irrigated with 2.4 cm of water. Throughout both the manure application and irrigation event, discharge, sediment concentration and water chemistry were monitored at the tile outlet, upstream reference site and three downstream sites. Data from the field studies were then used in the MOBED model to predict the flow hydrographs and bed shear stress distributions at a number of transects in the receiving stream. Using these predictions and the transport parameters that were obtained from rotating flume experiments with tile drain sediments, transport characteristics of the tile drain sediment in the receiving stream were predicted.

(3) Perform analysis of data collected from the rotating flume experiments on the transport characteristics of sediment from the storm water management pond in Kingston (Collaboration with Dr. Jiri Marsalek).

The transport characteristics of the deposited sediment from the storm water management pond in Kingston, Ontario were studied in the rotating circular flume. Both the deposition and the erosion processes were studied. The deposition experiments involved placing a known amount of sediment in the flume and operating the flume at different speeds to simulate different flow conditions. At each speed, the concentrations of sediment in suspension and the size distributions were monitored as a function of time during the course of the experiment. From these data, the critical shear stress for deposition and the amount of sediment that would stay in suspension permanently were established.

The re-suspension potential of the deposited sediment was also studied using the rotating flume. For these tests, the sediment was allowed to deposit on the flume bottom over a known period of time at a shear stress slightly below the critical shear stress for deposition and then the erosion characteristics were studied by applying the bed shear stresses in step increments. At each step the concentration of the eroded sediment and its size distribution were measured as a function of time. From such results, we can estimate the shear stress at which the sediment begins to erode, i.e. the critical shear stress for erosion for the surface layer of the sediment deposit. The erosion tests also provided quantitative data on the amount of sediment that would be re-suspended for a given bed shear stress. From these data empirical relationships were developed to evaluate the sediment deposition and erosion as a function of bed shear stress and these relationships can be used in mathematical models of fine sediment transport in the pond.

(4) Test and calibrate the instruments to measure the suspended sediment concentration and the flow field in the in-situ erosion flume.

A new in-situ erosion flume has been constructed to measure the in-situ bed shear strength of the cohesive sediment deposits in the field. The flume can be deployed over a sediment deposit and the critical shear stress for erosion of the sediment bed can be measured. It consists of a closed channel with a bottom opening to expose the sediment bed to the channel flow. The flow is generated by a submersible pump, which pumps water through the channel and discharges to the ambient water body. The bed shear stress exerted by the flow is calculated using a measured velocity at a single point in the flow field with a pitot tube and pressure transducer assembly and using the knowledge of flows fields in closed ducts. The erosion of sediment is monitored by measuring the sediment concentration in the water column in the channel using an optical device called "OSLIM". The testing and calibration of both the pitot tube assembly and the OSLIM are underway.

STUDY TITLE: Sediment/ Contaminant Transport in Rivers

STUDY LEADER: Y.L. Lau

STUDY TEAM: I. Droppo, J. Heidt, D. Liu, P. Engel

(1) Overview

Research was conducted into the erosion characteristics of contaminated bed sediments from Hamilton Harbour, the persistence of Irgarol as well as its occurrence in the aquatic environment. Experiments were carried out to investigate the effect of biofilm growth on the erodibility of bottom sediments and the effect of bed slope on the movement of coarse sediments. A study on the feasibility of biofiltration for removing contaminants from urban runoff was initiated.

(2) Erosion characteristics of coal pile deposits

The Kenilworth boatslip in Hamilton Harbour receives direct industrial discharges (primarily from steel manufacturing), combined sewer overflows and storm water 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 the eventual contamination of cleaner areas of the Harbour.

Experiments on the erosion characteristics of bed sediments were conducted in the 2-metre as well as the 5-metre annular flume. Sediments from the Kenilworth boatslip as well as kaolinite clay were used as bed material. It was discovered that the critical value of shear stress at which the bed began to erode was not a constant value but was dependent upon the conditions under which the bed was deposited. 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.

(3) 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. The results are presently being assessed.

(4) Persistence of Irgarol in the Aquatic Environment

Irgarol is a newly developed herbicidal additive for use in copper-based antifouling paints. It is intended to replace the antifouling agent tributyltin which may be banned internationally by 2003. However there is no information in the literature on the persistence and degradation of Irgarol and this hinders the assessment of its ultimate impact on the environment. A study was conducted into its persistence and degradation pathways. Photodegradation studies showed that Irgarol was very stable in distilled water and buffered solutions. However, Irgarol could be rapidly degraded when dissolved in natural waters (sea water and river water) and exposed to sunlight. Apparently, certain unknown organic photosensitizers in natural waters were involved in the accelerated photodegradation of Irgarol 1051. Three photodegradation products were also isolated, with one identified as M1 (2-methylthio-4-*tert*-butylamino-6-amino-s-triazine) which had been reported as a biodegradation product as well as a product of mercuric chloride-catalyzed hydrolysis.

Although Irgarol is generally considered to be non-volatile, there are no data in the published literature on its volatility. Experimental results from this study show that Irgarol 1051 cannot always be considered as non-volatile. When the temperature is above 35 °C and/or the water motion is rapid, significant Irgarol losses can occur through volatilization. These results will assist in the assessment of the fate and pathway of Irgarol 1051 in the aquatic environment.

This work generates new knowledge about the environmental behaviour of Irgarol 1051 and will be useful in development of water quality guidelines for this important antifouling chemical.

(5) 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. Calibration experiments had been completed and tests are continuing.

STUDY TITLE: *Characterisation of Flocs and Floc/Contaminant Associations***STUDY LEADER: G.G. Leppard**

This study covers many facets of research on flocs. The topics are: structure, composition and function relationships in microbial flocs; aquatic contaminant transport; transformation of contaminants after burial; particle/contaminant associations; new technology development for particle analysis and colloid characterisation; bacteria/heavy metal interactions; development of electron-optical analytical facilities. Major research and development activities are summarised briefly below. Our work on PAH contaminant transport in Hamilton Harbour was extended to include other contaminants and to yield greater amounts of isolated size fractions of contaminant-bearing flocs for characterisation. The additional contaminants studied were PCBs, organochlorines and heavy metals. The yield per particle fraction was increased tenfold. Research on methods for the characterisation of colloidal organic carbon (COC), and COC associations with contaminants, was expanded to include atomic force microscopy and X-ray absorption spectroscopy (XAS). Previous methodological successes for electron-optical characterisation of colloids and microflocs in surface waters, both fresh and marine, were transferred to the analysis of particle transport in groundwater. Transformations of aquatic particles after burial were analysed effectively with new correlative technology employing Scanning/Transmission Electron Microscopy coupled to Energy Dispersive Spectroscopy (STEM-EDS) jointly with XAS using a synchrotron (the Advanced Photon Source of the Argonne National Laboratories in Chicago). Using STEM-EDS alone, contaminant transformations in sediments were analysed to yield new information on accumulation and transformations of heavy metals by native sediment bacteria, with an emphasis on copper-contaminated lakes near Sudbury. With new preparatory technology and EDS quantification approaches, individual bacteria were analysed (hundreds of them, but one at a time), and so was the metal accumulation activity of given individual compartments within a single bacterium. Using EDS with the synchrotron-based technique, and sediments from a metal-contaminated pond near Chicago, the transformations could even be analysed on a "per particle/per colloid" basis for chemical bond changes involved in transformations.

In government/university/industry joint research covered under our NSERC Strategic Grant framework, we had considerable success in elucidating between the structure, composition and function relationships in microbial flocs, with some of the research being extended to novel compartmentalisation analyses of contaminated flocs (with silver & lead) and biofilms. The relationships between the bound water content of activated sludge and important engineering parameters (sludge retention time, floc structure and floc surface properties) were established. The biofilm results revealed a greater integration of biofilm components with industrial membrane structure than previously realised.

The international government work (Italy), on planetary scale problems, led to new monitoring protocols being introduced to the analysis of colloids and flocs in the Mediterranean Sea. One such planetary scale problem is the "mucilage phenomenon", a sporadic but massive reverse sedimentation (or flotation) of flocs in seas and oceans, leading to fouling of beaches and closure of fisheries on a grand scale. The 1989 example in the Adriatic Sea, where work was completed to uncover the mechanism of the phenomenon in the mid-1990's, led to an economic loss to the Italian tourist industry of \$ 4,000,000,000 during one summer

STUDY TITLE: Agricultural Non-point Sources of Pollution and Sustainable Agriculture

STUDY LEADER: H.Y.F. Ng

Research was completed on assessing nitrate concentration and loss in tile drain water, plant leaf transpiration and conductance, soil water content, water table depth, and tile drain volume, under the influence of controlled drainage and subsurface irrigation (CDS). Work continues on analyses of nitrate and tile drainage data collected from the Elora Research Station and nitrate and phosphorus in tile drainage water monitored from Bicrel, Chevalier and Shanahan experimental sites to determine nitrogen level and nitrate leaching and phosphorus transport in soil columns and in surface runoff under the influence of CDS, and tillage practices to determine agronomic and the environmental benefits. Major activities are summarized below:

- (1) *Assessment of tile drainage water quality on Bicrel, Chevalier and Shanahan experimental sites in collaboration with Agriculture and Agri-Food Canada (AAFC) researchers in Harrow.*

The 1996/97 data from Bicrel farm were analysed for soil moisture content, water table depth, plant leaf transpiration and conductance, tile drain volume, nitrate concentration and loss in tile drain water, under the influence of CDS. The results indicated that CDS increased soil moisture content by 15%, raised water table by 61%, increased tile drain volume by 20%, reduced cumulative nitrate concentration and loss respectively, in tile drain by 77% and 50% as compared with the free drainage. The CDS also increased the rate of plant leaf transpiration and conductance, respectively, by 33% and 11% as compared with free drainage. The yield of corn on the CDS plot had increased 64% as compared with the yield from the free drainage plot. The results of this study demonstrated that CDS had improved crop yields and reduced nitrate concentration and loss in tile drain water.

- (2) *Monitoring of surface meteorological parameters including UVB, PAR, soil temperature and moisture, water table on Bicrel, Chevalier and Shanahan experimental sites (in collaboration with AAFC researchers*

During the planting season of 1998, the Bicrel and Chevalier farms were ploughed by conventional method where no-till practice was applied on Shanahan farm. The soybeans, Northrup King 2492 were seeded on May 19 on the Chevalier farm and on May 24 on Shanahan farm. The soybeans, Pioneer 90205 were seeded on Bicrel farm on May 26. The crop yields from the plots under controlled drainage and free drainage for Chevalier and Shanahan farms, respectively are 4.75 t/ha and 4.63 t/ha and 4.70 t/ha and 4.39 t/ha. The yields from plots under CDS and free drainage for Bicrel farm, respectively are 4.53 t/ha and 4.52 t/ha. Although the crop yields shown in plots under controlled drainage and CDS are not significantly higher compared with the plots under free drainage, the improvements of crop yield are consistent. Data analysis on the effects of climatic conditions, tillage practices, soil type, soil nutrients and nutrients input to the crop yields and nutrient loss continues.

The surface meteorological parameters, UVB, PAR, soil temperature and soil moisture on Bicrel site were measured at 10 minute intervals. The data were reduced to hourly and daily values and were added to the existing MS ACCESS database that was created in 1997/98 fiscal year. Due to a very dry summer, there was no measurable amount of rainfall and subsequently there was no runoff and tile drainage water samples collected for a period from early June until late October, 1998. The water samples collected prior to June and after October were processed and submitted to NLET for chemical analysis and results are pending. Work continues on analysis of the tile drainage water

quality data that were collected in 1997/98 to determine the influence of climatic factors and tillage practices on tile drainage water quality.

(3) Monitoring nitrogen on plots located at Elora Research Station in collaboration with Professor R. Rudra, University of Guelph.

There was no measurable amount of rainfall and subsequently there was no tile drainage samples collected for a period beginning from early June to late October, 1998 because of a very dry summer. The tile drainage and rainwater samples collected prior to June and after October were processed and submitted to NLET for analysis of chemical compounds and results are pending. The hourly soil moisture and temperature monitored on the plots were processed and were added to the existing Elora database, that was created in 1997/98 fiscal year. Work continues on analysing data collected in 1997/98 fiscal year to determine the levels of nitrogen with no fertilizer input on the plots.

(4) Long term fertilization and crop rotation experiment in collaboration with AAFC researchers in Harrow at the Woodslee station of Agriculture and Agri-Food Canada.

The aim of this study is to investigate the efficiency use of fertilizers. The tile drainage sampling equipment were established by AAFC researchers and are operational. There was no tile drainage sample collected prior to June and before November, 1998 due to persistent dry weather. Work continues to establish a surface runoff facility to collect surface runoff samples draining from the long term plots and to install a rainwater sampler within the vicinity of the studied plots. A rainwater sampler has been purchased and outfitted for field installation. Installation of the rainwater sampler was postponed because of extended illness.

Sources and Fate of Toxic Substances Project

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. Current activities are in the areas of analytical methods development, environmental occurrence, persistence and fate of high priority chemicals such as tributyltin, nonylphenol and other "endocrine disruptors", polynuclear aromatic hydrocarbons, explosives, amines used in natural gas plants and new agricultural and antifouling pesticides. Collaborative research is done with others to assess the effects of these and other priority chemicals.

Source et devenir des substances toxiques

Ce projet permet d'obtenir d'importants renseignements scientifiques sur la persistance et le destin des composés chimiques toxiques dans les écosystèmes aquatiques. Les résultats de la recherche contribuent aux activités d'Environnement Canada reliés à l'évaluation et à la gestion des risques dus aux produits chimiques toxiques relargués dans l'environnement aquatique. Le ministère applique ces connaissances dans ses processus de prise de décision aux termes de la Politique de gestion des substances toxiques (PGST), de la *Loi canadienne sur la protection de l'environnement (LCPE)*, de la *Loi sur les produits antiparasitaires (LPA)*, des programmes sur les écosystèmes régionaux importants et sur les indicateurs de la santé des écosystèmes. Parmi les activités principales on retrouve: le développement de nouvelles méthodes d'analyse chimique, des études sur l'incidence dans l'environnement, la persistance et le destin des produits prioritaires tels que le tributylétain, le nonylphénol et autres substances dites "perturbatrices des fonctions endocriniennes", les hydrocarbures aromatiques polynucléaires, les explosifs, les amines utilisés dans la production du gaz naturel, les nouveaux pesticides utilisés en agriculture ou pour protéger les installations maritimes. Cette recherche se fait de manière concertée, dans des équipes pluridisciplinaires incluant souvent des chercheurs de l'extérieur.

STUDY TITLE: Fate and Effects of Pesticides and Industrial Chemicals in Water

STUDY LEADER: R.J. Maguire

STUDY TEAM: S.P. Batchelor

Research was initiated on the effects of tributyltin on freshwater benthic invertebrates such as *Hyalella azteca*. This work is being done by A. Bartlett, a Ph.D. student, under my supervision, that of U. Borgmann of AERB, and that of D.G. Dixon of the University of Waterloo. Chemical analyses are done in my laboratory. Research of a similar nature on freshwater snails (*Physa* sp.) continued with R. MacLean, a Ph.D. student (now resigned) with the University of Guelph. In addition, a review was made of the persistence of nonylphenol and its ethoxylates as part of the CEPA PSL2 assessment of these substances.

(1) Persistence of nonylphenol and its ethoxylates

Alkylphenol ethoxylates, in particular nonylphenol ethoxylates, are widely used nonionic surfactants that are discharged in high quantities to sewage treatment plants, and directly to the environment in areas where there is no sewage or industrial waste treatment. Nonylphenol ethoxylates can be biologically degraded in sewage treatment plants and in natural environments. Some of the degradation products, including nonylphenol, are more persistent than the parent surfactants, and they are found in receiving waters of sewage treatment plants. Nonylphenol in particular is found at high concentrations in some sewage sludges that may be spread on agricultural lands. While some sewage treatment plants discharge significant amounts of nonylphenol ethoxylate degradation products in their final effluents and digested sludges compared to what enters the plant, others degrade nonylphenol ethoxylates more or less completely. The differences in treatment efficiency of such compounds, and their degradation products, amongst different sewage treatment plants have been attributed to the load of the surfactants in influent streams, plant design and operating conditions, and other factors such as temperature of treatment. The highest nonylphenol ethoxylate elimination rates were achieved in plants characterized by low sludge-loading rates and nitrifying conditions. In natural waters, it appears that parent nonylphenol ethoxylates are not persistent, but some degradation products may have moderate persistence, especially under anaerobic conditions. Recent results from mesocosm experiments indicate moderate persistence of nonylphenol in sediments, with half-lives of 28 to 104 days. Microbial acclimation to the chemicals is an important determinant of persistence vis-à-vis biodegradation. Sunlight photodegradation of such products is also likely important. Further research on the persistence in natural environments of the lower ethoxylate and carboxylate degradation products, as well as nonylphenol, is necessary. Based on the limited data available, nonylphenol and the lower ethoxylates and carboxylates are persistent in groundwater. They are also persistent in landfills under anaerobic conditions, but they do not appear to be persistent in soil under aerobic conditions. Further research is required in order to more fully characterize the treatability of nonylphenol ethoxylates and their degradation products in sewage treatment plants, and their persistence in the natural environment.

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

STUDY LEADER: D.T. Bennie

STUDY TEAM: C. Sullivan, P. Low (contractor)

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

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

Studies to determine the environmental occurrence and fate of nonylphenol and its polyethoxylates continued with the collection and analysis of samples of various effluent streams of municipal sewage treatment plants and textile mills. Monthly sampling at two tertiary sewage treatment plants (STPs) was completed. Results of a small comparative study of 10 municipal STPs in Ontario are also complete as are the results of effluent sampling at a number of Ontario and Québec textile mills. These studies address research needs identified for the CEPA PSL 2 assessment of nonylphenol and its ethoxylates as well as departmental issues regarding endocrine disrupting compounds.

The alkylphenolic parameters determined in each study were 4-nonylphenol (4-NP), nonylphenol ethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO), nonylphenoxyacetic acid (NP1EC), nonylphenoxyethoxyacetic acid (NP2EC), nonylphenol polyethoxylates (NP_nEO, where n = 3 to 17), 4-*tert*-octylphenol (4-*t*-OP), octylphenoxyacetic acid (OP1EC) and octylphenoxyethoxyacetic acid (OP2EC). The sum of these parameters is referred to as total alkylphenolics (T-APE).

(1) *The occurrence, fate and effects of alkylphenol polyethoxylate surfactants in municipal sewage treatment plant effluents.*

Twelve month sampling programs were completed at the Guelph, Ontario and Edmonton, Alberta Gold Bar treatment plants. Both plants are tertiary treatment facilities but each utilizes different treatment and disinfection technologies. Total alkylphenolics in the influent at the Edmonton Gold Bar plant were quite variable and ranged from 110 to 1500 µg/L while levels in the final effluent were generally about 100 µg/L. Nonylphenol polyethoxylates (NP_nEO) were the major constituent of the Edmonton influent (71 - 97%). The major constituents of the Edmonton final effluent were the alkylphenoxy acetic acids which comprised from 85 to 97% of the discharged T-APE. At the Guelph facility, influent levels of T-APE ranged from 95 to 630 µg/L and effluent

levels ranged from 8 to 98 µg/L over the one year sampling period (n = 12). Nonylphenol polyethoxylates in Guelph influent were quite variable, ranging from 39 to 93% of the T-APE while the major constituents of the final effluent were the alkylphenoxy acetic acids. These comprised from 60 to 96% of the discharged T-APE in the effluent from Guelph. The most prevalent constituent of the sludge was 4-NP which comprised 34 to 96% of the T-APE in Edmonton samples and 29 to 94% of the T-APE at Guelph. The 4-NP concentrations in sludge ranged from 38 to 790 µg/g at Edmonton and from 65 to 380 µg/g at Guelph. Interestingly, the polar analytes making up NP(3-17)EO were the second largest T-APE component in the sludge. Concentrations in Edmonton sludge samples ranged from 28 to 57 µg/g while Guelph sludge values ranged from 55 to 115 µg/g. Both plants exhibited very low effluent concentrations of 4-NP and 4-t-OP (< 1 µg/L) and there did not appear to be any significant differences due to the technology used or climate. The only anomaly appears to be an increase in the effluent concentration of 4-t-OP which was noted during the winter months (November to April) at both plants.

Results of analysis of alkylphenolics and other endocrine disrupting chemicals at the various STPs across Canada have not been received yet from the company that was contracted to perform the sampling and analysis. All sampling has been completed.

(2) The occurrence of alkylphenol polyethoxylate surfactants and their degradation products in textile mill effluents.

Textile mills are significant sources of alkylphenolic substances generally and nonylphenol polyethoxylates, in particular. This study was carried out to determine the alkylphenolic loadings discharged from textile mills to sewage treatment plants. Sampling at 8 mills in Ontario and Québec showed very high levels of nonylphenol ethoxylates being discharged into sewers for treatment at local municipal STPs. Levels ranged from 190 to over 8000 µg/L for NP(3-17)EO and from 4 to 500 µg/L for NP1EO and NP2EO. Concentrations of alkylphenol carboxylic acid metabolites were very low, with most values being non-detectable. All 4-t-OP results were below 1 µg/L while 4-NP values ranged from 0.23 to 26 µg/L.

(3) The occurrence of alkylphenol polyethoxylate surfactants and their degradation products in the effluents and sludge of 10 Ontario municipal sewage treatment plants.

Results of the small comparative study of 10 municipal STPs in Ontario showed that secondary and tertiary treatment processes will have to be studied more carefully to ascertain if the elevated levels of alkylphenol carboxylic acids in final effluents and alkylphenols in digested sludge can be reduced. Alkylphenol levels in final effluents were below 1 µg/L with the exception of 2 facilities - one primary treatment only plant and one secondary treatment plant. Concentrations of alkylphenoxy acetic acids in final effluents ranged from 7 to 75 µg/L of NP1EC, 11 to 45 µg/L of NP2EC, 1.4 to 10 µg/L of OP1EC and 2.1 to 13 µg/L of OP2EC for secondary and tertiary plants. The only tested primary plant had much lower alkylphenoxy acetic acid results ranging from 0.15 to 1.6 µg/L. Concentrations of 4-NP in digested sludge are also a cause for concern with values ranging from 43 to 443 µg/g while 4-t-OP values ranged from 1.7 to 9.5 µg/g. Treatment method did not seem to affect these parameters for sludge samples.

STUDY TITLE: Priority Polycyclic Aromatic Compounds in Energy Production and Use

STUDY LEADER: B.G. Brownlee

STUDY TEAM: G.A. MacInnis

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 and T. Satchwill and E. Hargeshimer of the City 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 employed 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. 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.

A Tier 1 risk assessment of the PACs in the base/neutral extract was conducted based on the Ames testing results (R. Madill, M.Sc. Thesis, University of Guelph, 1998) using benzo[a]pyrene (BaP) equivalents (BEQ). The BEQ for porewater was 0.14 µg/L of BaP calculated from the concentrations of PACs quantitated and their relative mutagenic potential. Ames testing of the concentrated porewater base/neutral extracts resulted in no detectable mutagenic response with tester strain TA98 (detection limit 0.99 µg/L of BaP) or tester strain TA100 (detection limit 6.3 µg/L of BaP). Under a worst case scenario (pure porewater, no degradation of PACs) these results showed no evidence of risk to the wet landscape strategy for managing mature fine tailings from oil sands processing. A paper has been submitted for publication comparing the Ames test with the Mutatox® genotoxicity assay for assessing PACs in porewater.

In a parallel study, 13 PACs were tested for genotoxicity in the Mutatox® assay. One of the objectives was to determine if the mode of delivery of test compounds to the assay mixtures had an effect on the results since many of these compounds have a low aqueous solubility. To do this one set of tests was run using the standard assay procedure wherein the test compound was serially diluted in test medium. For a second set, serial dilutions of the test compound were first done in an organic solvent (methanol) and these dilutions were added to the assay mixtures. There were no detectable differences between the two approaches.

Both non-oxygenated and oxygenated PACs were tested without and with (S9) metabolic activation. In general, non-oxygenated PACs are inactive or weakly active with S9 activation and are strongly active without activation. Oxygenated PACs tended to be inactive both with and without S9 activation.

(2) Polycyclic Aromatic Compounds in Urban Runoff

Analysis of PACs in benthic invertebrates collected from areas receiving urban runoff is a useful means of assessing the fate of PACs in receiving waters and to assess potential impacts on biota. One of the challenges is in being able to analyze relatively small samples of biota (0.5-1 g wet wt) since separation of invertebrates from sediment is very time consuming. Work has been initiated to develop a method to analyze an expanded set of PACs (more than thirty) in small samples. Earlier work on toxicity testing of urban runoff samples is being published.

(3) Taste and Odour Compounds in Water Supplies

Work with J. Ridal of the St. Lawrence Institute of Environmental Sciences continued on the occurrence of two taste and odour compounds, geosmin and 2-methylisoborneol, in eastern Lake Ontario and the upper St. Lawrence River. A paper on earlier aspects of the work will appear in early 1999 (Ridal et al. 1999). A preliminary study on the aquatic toxicology of geosmin and 2-methylisoborneol was conducted by F. Gagné and C. Blaise, Centre Saint-Laurent.

In collaborative work with S. Watson and E. McCauley of the University of Calgary and T. Satchwill and E. Hargesheimer of the City of Calgary, headspace solid phase microextraction (SPME) has been used to analyze algal cultures and surface waters for odourous aldehydes (Satchwill et al. 1998; Watson et al. 1998). The use of headspace SPME resulted in improved detection limits and specificity over solution SPME previously used (Watson et al. in press). The headspace SPME technique was also applied to analysis of the common odour compounds, geosmin and 2-methylisoborneol, in raw and finished drinking water with detection limits of ca. 1-2 ng/L which is below the odour threshold concentrations of 10-20 ng/L.

STUDY TITLE: Broad Spectrum Detection of Toxic Substances and Transformation Products

STUDY LEADER: J.V. Headley

STUDY TEAM: K. Peru

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

(1) Natural wetlands and degradation of heavy-oils.

A report of an investigation of four wetland sites in Alberta was completed. Results obtained to date, indicate that natural wetlands may offer a cost-effective means for removal of amines, gas condensates and heavy-oil hydrocarbon mixtures. In support of field investigations, an ion-exchange liquid chromatography/ tandem mass spectrometry procedure was developed for conclusive confirmation of the uptake of diisopropanolamine in a variety of wetland vegetation. Complementary GC/MS procedures were also developed for the confirmation of plant-uptake of sulfolane. Work was also commenced on a new initiative "Assessment of Natural and Anthropogenic Impacts of Oil Sands Contaminants Within Northern River Basins". Preliminary field and laboratory studies were completed to characterize oil sands-derived hydrocarbon contaminants in sediments and water in the Athabasca River basin.

(2) Fate of gas-condensate in contaminated groundwater.

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

(3) Transformation of pesticides and toxic substances in biofilms.

Work was completed to measure the uptake kinetics of a broad range of pesticides in riverine biofilm. This research work was a collaborative venture with researchers at GKSS, Germany. This work indicated that the partitioning of contaminants is not limited to lipophilic regions of biofilms. Studies were conducted to investigate the mechanisms involve in the Photo-oxidative Degradation of N-methylpyrrolidinone in the Presence of $Cs_3PW_{12}O_{40}$ and TiO_2 Colloid Photocatalysts. Results obtained to date indicate that the combination of photocatalysed oxidative degradation in series with microbial degradation can be a more efficient means for destruction of amines than the single treatment of contaminants using either biofilms or photocells. Studies are continuing to evaluate the efficacy of photocatalysis combined with biofilm uptake for the attenuation of organic contaminants.

STUDY TITLE: ***Occurrence of Priority Organic Chemicals in Environmental Samples and Effluents***

STUDY LEADER: H.-B. Lee

STUDY TEAM: T.E. Peart

A comprehensive survey of nonylphenol ethoxylates and their metabolites in Canadian pulp and paper mill effluents and sludge has been completed. Research in the development of new or improved analytical methods for the determination of environmental estrogens is continued. A study on the occurrence of bisphenol A in environmental samples has been initiated. A study on the biodegradation of estradiol under aerobic and anaerobic conditions has also been completed. Major findings are listed below:

(1) Nonylphenolic compounds in pulp and paper mill effluents and sludge.

A survey of nonylphenol ethoxylates (NPE) and their acidic metabolites nonylphenol (NP) and carboxylic acids (NPEC) in pulp and paper mill effluents and sludge has been completed. Through the coordination of the Pulp and Paper Research Institute of Canada, a total of 19 Canadian and one U.S. mills of various process and waste treatment types provided primary and secondary treated effluents as well as sludge samples for this study. NPE and NP were found in many pulp and paper mill effluent samples, with concentrations mostly in the same order of magnitude (i.e. low $\mu\text{g/L}$) as the final sewage effluent. However, their levels in the secondary treated effluents were much lower than those observed for similar samples in an American study. In contrast, NPECs were not a major nonylphenolic component in the secondary effluent since they were found in only a few samples in this work. NPE and NP were also present in nearly all pulp and paper mill sludge, yet their levels (in low $\mu\text{g/g}$) were again much lower than those found in digested municipal sludge. Because of the complexity of the pulp and paper production, no trend between the surfactant levels in the effluent and the process and waste treatment types was observed.

(2) Analytical methodologies for endocrine disrupting chemicals (EDC).

Research in new or improved analytical methods for the determination of environmental estrogens is continued. A solid-phase extraction and GC/MS method is being developed for the determination of bisphenol A (BPA) in sewage effluents based on a concentration factor of 1000, the detection limit for BPA is 5 ng/L. Currently, this method is also being validated for the simultaneous extraction of other endocrine disrupting chemicals such as NP, NPE, and NPEC. The method previously developed for estradiol, estrone and estriol can now be extended for the determination of several other metabolites, including 16 α -hydroxy-estrone, which was found in many sewage samples. Attempts to lower the detection limits of estradiol in sewage samples by using negative ion chemical ionization mass spectrometry have been made. While this method provided a better than 50-fold increase in full scan sensitivity over the EI/MS technique, it may not be suitable for the detection of the PFP derivatives of estradiol and most of its metabolites due to the lack of characteristic ions required for selected ion monitoring work. Methods development work for EDC will be continued in the FY99/00.

(3) Occurrence of EDC in environmental samples.

A collaborative study between NWRI and the City of Toronto on the occurrence of NPE, NP, and NPEC in influent, effluent, and sludge samples collected from the four sewage treatment plants in Toronto has been completed. Data collected will be used in the supporting document for the CEPA assessment of NP. At the same time, data on the occurrence of BPA in sewage and pulp and paper mill effluent as well as sludge samples are being collected. Preliminary results indicated ubiquitous occurrence of BPA with concentrations in the low $\mu\text{g/L}$ (effluent) and low $\mu\text{g/g}$ (sludge) in the above mentioned samples. Work on the occurrence and fate of BPA in the Canadian environment will be continued in the next FY.

(4) Biodegradation of estradiol.

A study (with D. Liu) on the biodegradation of estradiol under aerobic and anaerobic conditions in the presence of an activated sludge was carried out. Under aerobic conditions, estradiol degraded rapidly into estrone as the principal metabolite, which further degraded into unknown compounds. Biodegradation of estradiol under anaerobic conditions followed a similar pattern, except at a much slower rate.

STUDY TITLE: Biodegradation of Priority and Targeted Chemicals

STUDY LEADER: D. Liu

STUDY TEAM: G.J. Pacepavicius, R.J. Maguire, Y.L. Lau, H.-B. Lee

Research was completed on the national survey for Irgarol 1051 in Canadian and Japanese aquatic environments, and the factors/processes affecting Irgarol environmental persistence. Research on biodegradation of the new antifoulant Sea-Nine 211 is still ongoing. Major activities are summarized below:

(1) National survey on the occurrence of Irgarol 1051 in the Canadian and Japanese environment

A two-year survey was conducted in 1996-1997 to investigate the occurrence of Irgarol 1051 in Canadian and Japanese aquatic environments. A total of 6 large trade ports (Vancouver, Toronto, Montreal, Halifax, Mizushima, Kobe), 73 marinas and 13 fishery harbours were surveyed. Irgarol 1051 was not detected in the Canadian aquatic environment, but was positively identified in the enclosed coastal waters of the Seto Inland Sea in Japan. Among the 6 trade ports surveyed, only the Mizushima Port had low levels of Irgarol 1051, up to 19.5 ng/L. Approximately 27% of the marinas surveyed in the Seto Inland Sea were found to have been contaminated by Irgarol 1051, ranging in concentration between 12.5 and 264.2 ng/L. Interestingly, Irgarol was found more frequently in fishery harbours than in marinas, indicating that besides marinas and trade ports, fishery harbours can also be a significant source of contamination for the aquatic environment. Irgarol 1051 had not been found in fishery harbours before, and it is suggested that fishery harbours, in addition to ports and marinas, should be included in future Irgarol 1051 surveys. Irgarol 1051 has been reported to be highly toxic to non-target marine algae with observable growth inhibition at a concentration as low as 50 ng/L, which is well within the ambient concentration levels found in some localities of the Seto Inland Sea.

This work generates, for the first time, information on ambient concentration levels of Irgarol 1051 in aquatic environments outside Europe. Since such information is currently not available in the open literature, the results of this study have been communicated to Environmental Assessment Division of Pest Management Regulatory Agency for its consideration in the registration of Irgarol 1051 in Canada under the Pest Control Products Act.

(2) Factors/processes affecting the environmental persistence of Irgarol 1051

Irgarol 1051 is a new replacement chemical for tributyltin, which has been regulated internationally due to its severe impact on aquatic ecosystems. There is no information in the open literature on the photodegradation of Irgarol, a fact that hinders the assessment of its ultimate impact on the environment. This study showed that Irgarol was very stable in distilled water and buffered solutions. However, Irgarol could be rapidly degraded when dissolved in natural waters (sea water and river water) and exposed to sunlight. Apparently, certain unknown organic photosensitizers in natural waters were involved in the accelerated photodegradation of Irgarol 1051. Three photodegradation products were also isolated, with one identified as M1 (2-methylthio-4-*tert*-butylamino-6-amino-s-triazine). M1 had previously been reported as a biodegradation product as well as a product of mercuric chloride-catalyzed hydrolysis. Thus

Irgarol biodegradation and photodegradation processes in natural waters have that major degradation product in common.

This work generates important information about the persistence and abiotic degradation pathway for Irgarol 1051. Such information is currently not available in the open literature, thus hindering the hazard assessment for Irgarol 1051 as well as impeding the development of management strategies for the control of this chemical in the aquatic environment. Irgarol 1051 has been recently registered in the United States as a replacement biocide for organotins in antifouling paints, and is also being seriously considered for registration under Canada's Pest Control Products Act.

STUDY TITLE: Sources and Fate of Toxic Substances

STUDY LEADER: F.I. Onuska

STUDY TEAM: K. Terry, E.M. Veira

Research was completed on the survey for benzidines and pigments in industrial wastewater, sludge and finished water at a pigment-manufacturing plant and the Humber River Sewage Treatment Plant (STP) in Toronto, Ont. Data were provided to the Environmental Contaminants and Nuclear Program Division of Ontario Region to aid in the development of the Memorandum of Understanding between Environment Canada and the industry in question pursuant to the CEPA PSL1 Strategic Options Process for 3,3'-dichlorobenzidine. Research was also performed on explosives and microcystins. Major results are summarized below.

(1) The occurrence of 3,3'-dichlorobenzidine (3,3'-DCB) in industrial wastewater and municipal sewage treatment plant effluent and primary sludges

Aromatic amines including anilines and benzidines found in some industrial effluents could be a result of the biodegradation of the azo compounds used as pigments. These amines are known to be highly toxic and carcinogenic. These pollutants have been positively identified and monitored in a Toronto industrial plant effluent and the Humber River sewage treatment plant (STP) influent in Toronto, Ont. During last three years, we collected industrial wastewater samples and sewage in co-operation with Toronto Waterworks Department of the Municipality of Metropolitan Toronto. Temporal and spatial monitoring of effluents was carried out.

The temporal variation of their presence indicates that the average concentration for 3,3'-DCB has been varying between 0.2 to 650 µg/L. A finished water from the Humber River STP effluent pumped to Lake Ontario did not contain any detectable amount of 3,3'-DCB. A Humber River STP primary sludge did not show any aromatic amines.

It is presumed that the reductive cleavage of benzidine-based pigments is resulting in the formation of aromatic amines by bacteria in sewer lines and the STP. To gain a better understanding of 3,3'-DCB and aniline formation by these bacteria we set an experiment to study anaerobic and aerobic sludge-water laboratory biodegradation experiment. Preliminary results show that diarylide yellow pigments such as Pigment Yellow 83 cleaves under anaerobic and aerobic conditions to form free aromatic amines.

In addition, the thirteen different pigments manufactured in the plant in question have also been shown to contain 3,3'-DCB in the range of 11 to 270 mg/kg of 3,3'-DCB. The worst-case scenario calculations indicate that the pigments contained 3,3'-DCB represent approx. 157 kg of unreacted 3,3'-DCB in the total production in DCC1242-Yellow 83 pigment alone per year.

(2) Analysis of high energetic materials

Residues of high energetic materials such as TNT, RDX and HMX constitute a significant pollution problem at industrial manufacturing sites and military installations. These pollutants have caused groundwater pollution and significant contamination of sediment and soil that is reclaimed for agricultural usage.

In co-operation with the Defence Research Establishment at Valcartier, PQ and the National Research Council of Canada, Biotechnology Research Institute in Montreal, groundwater and industrial site pond sediment samples were collected. The groundwater samples contained TNT and 2- and 4-aminodinitrotoluenes and the pond samples were polluted with RDX and HMX. The groundwater samples were collected in May 1998 and contained 0.92 mg/L of TNT; the second sample collected from the same pond in November 1998 contained 1.22 mg/L of TNT and 2.1 mg/L of 2-amino-4,6-DNT. The pond sediment samples contained 12,300 mg/kg of RDX and 8,260 mg/kg of HMX.

Research was completed on the evaluation of extraction methodologies for determining explosives in water and sediments. It was concluded that the most reliable and cost effective method for extracting water is microextraction by demixing with acetonitrile. For sediment and soil samples the accelerated solvent extraction is the most suitable mean to achieve almost a theoretical recovery using acetonitrile.

(3) Microcystins

Due to adverse biological and human health effects of microcystins, there is a need for rapid analytical techniques that can provide qualitative and quantitative detection of the toxins in environmental samples.

We recently developed a method of determination using a capillary zone electrophoresis (CZE) technique called isotachopheresis (ITP) with UV-detection. In this preliminary study, the utility of ITP and ITP-CZE as a viable alternative for microcystins was based on selectivity, peak capacity, duration and cost for analysis. The results obtained demonstrate that ITP-CZE is an applicable method in the separation of microcystins. We can influence the migration time, elution order, baseline separation and selectivity of these analytes by modifying the composition of the buffer with organic solvents. This study will continue in the next FY.

STUDY TITLE: Environmental Effects and Fate of Metals and Organometals

STUDY LEADER: F. Yang

Research was carried out on (1) monitoring of butyltin compounds in marinas and their seasonal variations, (2) biomonitoring study with caged mussels at polluted harbour sites in southern Ontario (3) development of analytical techniques for the gasoline additives MMT (methylcyclopentadienylmanganese tricarbonyl) and MTBE (methyl *tert*-butyl ether) in aqueous and air samples. Major studies are summarized as follows:

(1) Monitoring of tributyltin (TBT) in marinas and its seasonal variations

The use of TBT in antifouling paints was regulated 10 years ago in Canada, but it still occurs in aquatic environments today. In order to find out the sources of TBT in water, the concentration of TBT in six marinas were monitored for nine months. The concentration of TBT in water was found to increase with the increasing boating activities in marinas. The concentration of TBT was up to 14 ng Sn/L in the summer, and lower than 2 ng Sn/L in the winter. Water samples at various depths in the middle of Lake Ontario were also collected, and TBT was only found in the water samples obtained from a depth of 0.5 m at two locations, and was not determined in deeper water samples. Surface sediment samples from marinas and Lake Ontario were also analysed. The results indicated that small amounts of TBT were released from antifouling paint and not from resuspended sediment.

(2) Biomonitoring for TBT with caged mussels at selected harbours in southern Ontario

The mussels (*Elliptio complanata*) were collected from Balsam Lake and were used to study the bioaccumulation of TBT. Caged mussels were placed in six selected harbour sites. Four mussels from each cage and 4L water samples were collected at each site monthly and analyzed for butyltin compounds. Another 100 mussels were used to study the uptake of organotin compounds in water spiked with TBT in the lab. Preliminary results showed that bioaccumulation of TBT in mussels was not only related to the exposure time but also related to mussel size.

(3) Determination of butyltin compounds in air

A research project on the determination of butyltin compounds in air, a collaboration between NWRI and the McMaster University School of Public Health, has been completed. TBT, dibutyltin (DBT) and monobutyltin (MBT) in air were successfully trapped with solid absorbent, then extracted by sodium diethyldithiocarbamate/toluene/pentylmagnesium bromide, and determined by gas chromatography with an atomic emission detector (GC-AED).

(4) MMT (methylcyclopentadienylmanganese tricarbonyl) in the environment

The use of MMT in gasoline is expected to increase in the future, and human health concerns have been raised recently. Methods have been developed and applied to determine MMT in water and air.

(i) Determination of MMT in aqueous samples by SPME-GC-AED.

Solid phase microextraction (SPME) is a simple, solvent-free and efficient extraction techniques. The combination of SPME with GC-AED provides a convenient and sensitive analytical method for the determination of sub-ppq levels of MMT in aqueous samples with good precision. Different kinds of aqueous samples were collected and extracted for the determination of MMT with the SPME technique. The liquid-liquid extraction method was also used for MMT determination in these samples and the results from the two techniques were comparable.

(ii) Application of solid phase extraction in the determination of MMT in air.

The solid absorbent sampling technique for MMT in air has been modified. The technique is convenient and efficient for field sampling, especially for larger volume sampling. This technique has been used for the determination of MMT in air in our lab before, and is being applied in collaborative research with the University of Montreal in its study on effects of exposure to MMT on human health.

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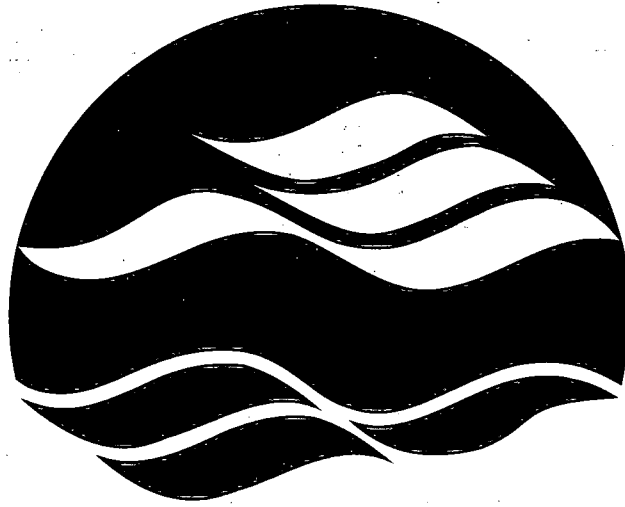
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