Toxic Substances Research Initiative

# Research Summaries of TSRI projects

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# Foreword

This document is a compilation of project research summaries funded under the Toxic Substances Research Initiative (TSRI). Researchers were requested to submit to the TSRI Secretariat, summaries of their research projects in a plain language format. All five priority research areas funded under the Initiative are included, they are: Persistent Organic Pollutants; Specific Forms of Metals in the Environment; Endo crine Disrupting Chemicals; Urban Air Quality and Exposure to Airborne Pollutants; and Cumulative Effects of Toxic Substances.

Also included under each priority research area is the type of information that was required by TSRI in order for research to be considered for funding. Applicants were asked to clearly demonstrate that the proposed research addressed both a priority research area and an identified priority knowledge need. All priority knowledge needs are provided for fiscal years 1999-2000 and 2000-2001. Please note that there were no call for proposals in fiscal year 2001-2002, therefore, no knowledge needs were developed for any of the priority research areas. There were, however, ongoing projects that were previously approved in fiscal year 1999-2000.

As stated, the majority of this document contains research summaries from the projects funded by TSRI since its launch in 1998. It should be noted that the summaries do not constitute the final findings for the majority of projects contained herein, they are only intended for use as a summary document.

Further information about the Initiative is available on the TSRI website located at www.hc-sc.gc.ca/tsri. Inquiries can be directed to: PM_TSRI-GP_IRST@hc-sc.gc.ca.

# Persistent organic pollutants (POPs)

POPs are chemicals which exist in the environment for long periods of time, can concentrate and accumulate in the food chain and can travel great distances through the atmosphere. Since its launch in 1998, the TSRI has allocated over $7.4 million in funding 21 research projects in the area of Persistent Organic Pollutants.

In fiscal years 1999-2000 and 2000-2001, applicants were asked to strengthen and accelerate the development of our scientific knowledge of POPs which may move long distances and cause significant effects on human health and the environment.

### In fiscal year 1999-2000, the priority knowledge needs were:

- To determine and link the ecosystem and human health effects of known and emerging issue POPs such as endosulfan; pentachlorophenol and pentachloranisole; short-chain chlorinated paraffins; triazines, chlordane and toxaphene;
- To determine the degree to which domestic and international sources are contributing to observed levels of POPs in Canada;
- To understand the impacts of POPs on human health outcomes (e.g. fetal development, the relationship between POPs and cancer in children and Aboriginal peoples, etc.);
- To develop the data necessary to determine ecosystem and human health risks associated with known priority POPs;
- To determine the long-range transport characteristics of known and emerging POPs;
- To complete the research needed for the development of new human tissue guidelines, blood guidelines, acceptable daily intakes, and health based advisories especially for at-risk populations such as children, pregnant women and Aboriginal peoples;
- To identify mechanisms of action for toxic effects seen in ecosystems and humans exposed to POPs where policy and/or regulatory decisions are required; and
- To improve understanding of pharmacokinetic and pharmacodynamic characteristics of POPs where regulatory decisions are required.

### In fiscal year 2000-2001, the priority knowledge needs were:

- To develop the data necessary to determine ecosystem and human health risks associated with known priority POPs from domestic and international sources, particularly in relation to the development of new human tissue guidelines, blood guidelines, acceptable daily intakes, and health based advisories especially for at-risk populations such as children, pregnant women and Aboriginal peoples; and
- To develop approaches to study the transport of POPs, particularly in relation to determining the degree to which domestic and international sources are contributing to observed levels of POPs in Canada and, in the case of international sources, their countries of origin.

### In fiscal year 2001-2002:

There were no call for proposals in fiscal year 2001-2002, therefore, no knowledge needs were developed for any of the priority research areas. However, there were still ongoing research projects which were previously approved in the 1999-2000 fiscal year.

# Determination of the impact of polybrominated diphenyl ethers (PBDEs) on Canadian environment and health of Canadians

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Flame-retardants are chemicals that are added to resins and polymers that are used in paints, textiles, electric and electronic equipment to prevent them from catching fire and to slow down the burning process. Therefore, these chemicals are incorporated in many commercial products intensively used in modern life (such as TVs, computers, cars, clothing, building materials, etc.). Flame-retardants are divided into two major subgroups; reactive flame-retardants that are a group of compounds that are chemically bonded to the plastics; and additive flame retardant compounds, which are only mixed together with the other components of the polymers. As a consequence, additive flame retardant compounds can be easily released from discarded components and find their way into the environment. The most frequently used additive flame retardant compounds are polybrominated diphenyl ethers (PBDEs), which have been produced in large quantities since the early 1980's. The annual production of PBDEs increased from 20,000 tonnes in 1984 to 67,000 tonnes in 1999. Due to rigorous fire regulations in many countries, the use of brominated flame-retardants such as PBDEs are expected to grow at about 5% annually. PBDEs are persistent and tend to concentrate in animal/human tissue. Anderson and Blomkvist first reported PBDEs in fish from Swedish rivers in 1981. Since then detectable levels of PBDEs have been reported in a number of environmental compartments around the world including pristine areas such as the Arctic, indicating PBDEs are ubiquitous environmental pollutants. PBDEs have also been detected in human tissue; a study by Norén and Meironyté showed a five-fold increase in levels of PBDEs in mothers' milk in Sweden over the past twenty-five years. These results prompted the European Union to ban the use of penta-BDE formulation as of July 2003.

In this study, we evaluated the impact of PBDEs on the Canadian ecosystem and the health of Canadians. Two major components of the research were, a) levels and trends in environmental compartments, b) toxicity. The initial focus was to determine the distribution of PBDEs in the Canadian environment. PBDEs were detected in all of the environmental compartments, including air (Arctic and Great Lakes), water (Lake Ontario), suspended sediment (Wapiti River), sediment (Lake Ontario, St Lawrence Estuary), sewage sludge (across Canada), biota (St Lawrence Estuary, Great Lakes, Arctic, Strait of Georgia), wildlife eggs (across Canada), food basket, and human milk (across Canada). The next phase of this study was to determine the temporal trends of PBDEs in archived biota, gull eggs, human milk and sediment core. These results indicated that the levels of PBDEs are on the rise in Canadian environment. The rate of increase in the levels of PBDEs correlated well with the increase in the use of bromine in the production of brominated flame-retardants. Toxicity studies indicated that PBDEs are less toxic than polychlorinated dibenzo dioxins and furans. However, recent results from Europe indicate that these compounds can interfere with thyroid hormone functions, and have some estrogenic activity.

# Reproductive/developmental effects of an environmentally relevant organochlorine mixture

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Several organochlorine compounds which are found as a complex mixture in aquatic food chains have been shown to interact with hormonal systems such as androgenic and oestrogenic systems. Our main hypothesis is that *in utero* and lactational exposure to an environmentally-relevant complex mixture of endocrine-disrupting organochlorines alters the development of the male reproductive tract, which in turn leads to adverse effects on male reproductive function during adulthood. Our secondary hypothesis is that this mixture of organochlorines modifies the maturation of gametes and early embryonic development. The pig was selected as the experimental model because of physiological similarities to humans especially with regard to reproductive and endocrine systems.

In the *in vivo* part of this study, sows were administered an organochlorine mixture similar to that found in traditional foods from the Arctic aquatic food chain, which are part of the diet of Inuit people living in Nunavik (northern Quebec, Canada). Sows were inseminated with the semen of an untreated boar and treatment was continued throughout gestation and lactation. Male pigs were nursed during their first month of life and received a standard diet after weaning. Prenatal exposure to the mixture caused piglets to be shorter at birth. Boars from the high dose group had reduced sperm motility compared to controls. Furthermore, a dose-response decrease in testicular weight was observed at autopsy, which may be indicative of an antiandrogenic effect.

Using various cell systems expressing luciferase as the reporter gene, the endocrine disrupting potential of our mixture was investigated. The mixture displayed anti-androgenic activity which appeared to be due solely to its content in *p,p'*-DDE. It also displayed a weak estrogenic activity that may be accounted for by xenoestrogens such as *p,p'*-DDT, β-hexachlorocyclohexane and *p,p'*-DDE. In addition, the mixture exhibited a dioxin-like activity similar to that induced by mono-ortho PCB congeners.

The last series of experiments involved *in vitro* exposure of porcine gametes to the mixture. Exposure of cumulus-oocytes complexes during *in* vitro maturation decreased the quality of cumulus cell expansion and increased the percentage of these cells undergoing programmed cell death (apoptosis). The mixture also decreased oocyte maturation and early embryonic development, following insemination with untreated boar sperm. Exposure during *in vitro* fertilisation decreased the rate of sperm penetration of the oocyte and early embryonic development. Incubation of pig sperm alone with the IVF medium containing the mixture reduced sperm motility and viability.

Our results indicate that *in utero* and lactational exposure this organochlorine mixture, which is similar to that found in the Arctic aquatic food web, can alter growth and reproductive organ development in male pigs. *In vitro*, the mixture disrupts oocyte and sperm fertility and further embryonic development. Additional studies are underway in the rat model to further test this mixture.

# Sources of Agrochemicals to the Atmosphere and Delivery to the Canadian Environment

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This study examined whether the continuing input of banned pesticides into the Canadian environment is due to recycling from existing contaminated soil and water, or due to atmospheric migration from use of these pesticides in countries other than Canada. The study sought to determine the source of airborne pesticides through surveying the agricultural soils in selected areas of Canada and the U.S in order to gain a better understanding of where airborne pesticides come from and how they are transported to Canadian ecosystems.

# Mechanism of gender related tumour promotion induced by hexachlorobenzene in the rat

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Hexachlorobenzene (HCB) is a widespread environmental contaminant which has been used as a fungicide and is a byproduct of certain industries. While the use of HCB as a fungicide has been banned in most industrialized countries for about 30 years, it is a persistent contaminant which is still present in the Canadian environment. Human exposure to HCB has been linked to the development of porphyria and hepatic cancer. Previous studies in rodents have shown that HCB is an epigenetic carcinogen, i.e. it does not cause DNA mutations. HCB administration to rats renders females more susceptible than males to develop hepatic tumors. Gap junctions are composed of integral transmembrane proteins termed connexins (Cx). There are two Cxs in the liver, Cx26 and Cx32. The objective of the present study was to determine the effects of HCB on gap junctional intercellular communication (GJIC), to characterize the sexual dimorphism in HCB-induced tumor formation. Short-term exposure of female rats to HCB resulted in a long-term decrease in GJIC. This decrease in intercellular communication in female rats appears to be the result of a decrease in the synthesis of both Cx32 and Cx26 that are the proteins responsible for forming the cellular pores that constitute the gap junction. In male rats the levels of Cxs are unaltered by HCB treatment. This decrease in Cx32 and Cx26 in females occurs prior to the formation of tumors, suggesting that this is an early marker of chemically-induced carcinogenesis.

Comparing gene expression using a cDNA array containing almost 1200 genes indicate that an important difference in the response of males and females to HCB exposure. In males gene expression, gene expression appears to be upregulated as compared to control rats. In female rats exposed to HCB, however, gene expression appears to be decreased for the majority of hepatic genes studied. This sexual dimorphism in the response of the liver may provide essential information towards understanding not only the sexual dimorphism of HCB-induced carcinogenesis, but may also provide novel understandings of hepatocarcinogenesis. This research will permit to improve the risk assessment of the health risk caused by HCB.

# Developmental neurotoxicity of environmentally-relevant mixtures of persistent organic pollutants

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There is scientific and public concern that exposure of fetuses, infants and children to environmental chemicals affects growth and development, and in particular, nervous system development and function. Both human epidemiological studies and laboratory testing with rats suggest that persistent organic pollutants (POPs) can alter development and brain function. Although the animal studies can show cause-effect relationships, they have often been conducted at very high doses or with single chemicals or simple mixtures that do not reflect true human exposure. This study was designed to evaluate the neurodevelopmental effects of exposure to a mixture of POPs that is based on blood levels in people who consumed large quantities of Great Lakes fish. To do this, groups of pregnant female rats were exposed to different doses (1 to 1000 times human blood levels) of the POPs mixture from the first day of pregnancy until weaning. The neurodevelopment of the offspring was evaluated at various ages to assess the persistence of effects and to uncover any potential delayed effects. A variety of measures obtained in juveniles, young adults and mature adults provide a comprehensive assessment of toxicological effects and evaluate biological effects that may mediate altered neurological functioning.

Although reproduction was not affected by the mixture, the highest dose of the chemicals decreased birth weights and growth rates and these effects persisted until at least 35 days of age. The most striking observation was the unexpectedly high mortality rate (80%) and the presence of abnormal facial structure in surviving pups of the highest dose group. These results were unexpected based upon previously published studies and our own pilot study, and suggest that there may be additive or synergistic effects of combined exposure to a large number of chemicals. These data also show the importance of conducting toxicological studies using relevant mixtures of contaminants rather than with individual chemicals.

Because behavior reflects the functional integration of the nervous system, a variety of behavioral tests were conducted in juvenile and adult animals. These tests provided measures of sensory and motor functioning, neural reflex development, reactivity, learning capacity and memory function. Results available thus far indicate that the mixture of chemicals does alter nervous system function although the full extent of these disturbances is not yet clear.

Other toxicological endpoints linked to behavioral measures have been collected and are currently being analyzed. Brain neurochemistry will provide measures of the impact of the POPs on chemical messengers in specific regions of the brain while neuropathology data will assess the impact of the POPs mixture on anatomical and cellular structure in the brain. Brain molecular biomarkers will provide data on cellular damage in the brain by measuring alterations in specific protein levels in the brain. We have also collected data on the systemic effects of exposure to the chemical mixture. Results from both the pregnant mothers and the offspring show that liver enzymes which help metabolize and detoxify foreign compounds are elevated but only after the highest dose of the mixture. The highest dose of the mixture also altered thyroid structure in mothers but thyroid hormone levels were not affected.

A unique feature of this research is the integration of the neurobehavior, neurochemistry, neuropathology, molecular and systemic effects in order to provide a comprehensive assessment of the neurodevelopmental impact of POPs following exposure to these chemicals before and after birth. The results generated will provide public health officials and the general public with information needed to better understand the neurotoxic health risks of early-life exposure to chemical mixtures.

# Male reproductive function and DDT in Chiapas, Mexico

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DDT continues to be applied against mosquitoes in several countries to control malaria. Its stability, persistence and widespread use have meant that DDT residues can be found everywhere and food-borne DDT remains the greatest source of exposure for the general population. The short-term acute effects of DDT on humans are limited, but long-term exposures have been associated with chronic health effects including fertility problems in males.

The findings from our pilot study constitute the first evidence that a high-DDE (major metabolite of DDT) body burden may alter hormonal status and reproductive function in men. The basic aims of this study were to assess biological exposure, hormone profiles, basic semen parameters, and sperm function, in order to determine the association between DDT exposure and male fertility in a large sample of Mexican males in Chiapas, one of the most spayed regions with DDT in Mexico (malaria control). One hundred and sixty one men in the Tapachula area in Chiapas have been recruited for participation in the project. Twenty-eight men were from a non-exposed community (19 with two and 9 with one sample) and 17 participants did not meet the inclusion criteria or were unable to give a semen sample. All participants gave blood samples. The 28 participants were organic coffee plantation workers, from an adjacent community, and might serve as a control group, as no DDT has been used in this area. A comprehensive questionnaire was applied, including sections on toxic exposure, diet, fertility and clinical history. The data were computerized and compared to fertility parameters, pesticide and steroid data.

The mean DDE concentration was found to be 180 µg/l.(ppb). When adjusted for total lipids the mean DDE concentration in serum lipids was 47.2 mg/kg (ppm). Both DDT and DDE were detected in the non-exposed group. The semen analyses revealed that the semen volume of these men was subnormal. The mean volume of 1.84 ml in the exposed group might give an indication of toxic involvement at the level of the seminal vesicles and prostate. The pH of the seminal plasma was found to be high, with a mean of 7.98. The low volume and high pH are both indicators of prostatic involvement. A positive correlation between DDT concentration and testosterone levels was found. The increase in testosterone levels is a direct indication of the anti-androgenic effect of DDT.

The fact that reproductive parameters and hormone status of the participants were affected has far reaching implications for human health. The results emphasize the need to closely monitor and further investigate populations chronically exposed to high doses of DDT for occurrence of reproductive effects.

# Accumulation of organochlorine contaminants by osprey

John Elliot

The osprey (*Pandion haliaetus*) is a piscivorous bird with a nearly worldwide distribution. It is sensitive to effects of chlorinated hydrocarbon contaminants (CHCs), such as DDE on shell quality and embryonic viability. Through the 1960s and 1970s, this led to declines in osprey across North America. With the ban of the most toxic of the CHCs, there have been overall population recoveries in many areas.

Hotspots remain, and a recent study showed elevated DDE levels in osprey eggs in the Pacific Northwest, 23% of which were at levels (>4.3 mg/kg) associated with developmental effects. Recent studies are also showing CHC concentrations in fish from high elevation lakes in BC and Alberta sufficient to pose a threat to breeding osprey. This contamination is due to atmospheric transport from areas of both current and historical use with subsequent deposition into cold regions, either north or at high altitude. Ospreys are exposed to DDT in wintering grounds in Latin America, where use of the insecticide continues. In this work, we have been studying osprey nesting in higher-elevation sites to determine their exposure to CHC contaminants and evaluate the relative contributions of nesting and wintering areas to their total body burden.

To do this, we have been measuring CHC residues in osprey egg, blood samples and in fish prey species in nesting areas throughout BC and into the southern Yukon. First, nest sites were found on water bodies either in alpine areas or receiving alpine drainage. A single egg was removed from a subset of the available nests, and analyzed for CHCs. Where possible, this was coupled with sampling and analysis of blood from adult female, and the data together provide an estimate of the CHC burden entering the nesting area. At 5-7 weeks, the nest was re-visited and a blood sample collected from the remaining nestlings to estimate the CHC burden accumulated from food. The effect of these CHC levels on nesting success was evaluated by a combination of egg and chick counts, and compared to local residue measurements. Fish prey species were collected and analyzed for CHCs in order to estimate the local food contribution. At some sites, satellite transmitters were attached to the osprey and their movements tracked to southern wintering areas. Fish collected and analyzed from southern areas provide an estimate of CHCs at wintering sites.

Fieldwork for this study is complete, but the chemical analyses are still underway and will not be available until early 2002. Preliminary results show detectable CHCs in all fish and osprey egg/plasma samples. Elevated DDE, at 18 ppm, was found in one bird from Exshaw Lake, Alberta, but all others have been less than 4.3 ppm. In most cases, CHC levels were low in both blood and prey in BC relative to southern exposures. Satellite transmitters were attached to fifteen birds through the study, most of which wintered in the Mexico in the states of Sinaloa and Veracruz, with one travelling to N Venezuela.

Results thus far are good news for osprey populations in BC, which seem not to be carrying DDE burdens which might affect reproductive successes. There is evidence, however, of high-elevation fish populations accumulating DDE levels which would be of concern to fish-eating wildlife, including osprey. Telemetry results show, for the first time, that ospreys from western Canada travel as far as South America to wintering grounds.

# Effects of agricultural pesticides on amphibian physiology

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It is well documented that, for some fifteen years, amphibians have been disappearing from many locations throughout the world. Several environmental factors are suspected as being the cause of this population decline, including habitat modification and/or destruction, UV radiation, infectious agents, parasites and chemicals. While all these factors may be acting alone, sequentially or in synergy, the one that receives the most attention is pollution.

The main objective of this project was therefore to develop and validate a laboratory amphibian model that would enable us to study the effects on amphibian physiology of exposure to pesticides of agricultural origin, at levels found in the environment. The research focused primarily on the endocrine, reproductive and immune systems. Two frog species were studied: *Xenopus laevis* to optimize the model, and *Rana pipiens*, as a species indigenous to Quebec. Two means of exposure were also used. The first contained a specific mixture of pesticides (atrazine, aldicarb, dieldrin, endosulfan, lindane, metribuzin) and was used in mechanistic studies; the second utilized water collected from a Quebec river (Chibouet) in early June, a time when pesticides are present in significant quantities. Finally, the last stage of the project consisted of field studies involving *Rana pipiens* living in clean and contaminated sites.

For 21 days, both species were exposed to the pesticide mixture at levels that were ten times lower than those found in the environment. This exposure produced significant changes in the immune system by affecting phagocytosis and suppressing lymphocyte proliferation. In terms of the reproductive system, this level of exposure resulted in significantly more shrunken testicles and an increase in the number of oocytes in females. With regard to the endocrine system, analysis of vitellogenin showed no significant changes. To complement the data on the immune system, we developed a resistance model for *Rana pipiens* for the parasite *Rhabdias ranae*. Each frog was exposed to the mixture for 21 days and was infected with approximately 30 *Rhabdias* larvae. The frogs were returned to aquariums containing unchlorinated water, and the infection monitored for 21 days. A much higher level of infection was noted in subjects with weakened immune systems.

With regard to exposure to the water from the Chibouet River, we observed a 30% increase in tadpole mortality and a significant decrease in phagocytosis. However, the most dramatic effect was a halt in the process of metamorphosis. At the beginning of exposure, the tadpoles had reached stage 45, and at the end of the 44 days of exposure, although they were larger, the tadpoles were still at stage 45. The reproductive system, which was very undeveloped at this stage, could not be studied.

The very important consequences observed in terms of metamorphosis and the immune and reproductive systems clearly demonstrate the vulnerability of amphibians to pesticides of agricultural origin. Whether these pesticides act by diminishing the resistance of animals, thereby making them more vulnerable to various types of infection, and/or by altering the development of the reproductive system, and/or by blocking metamorphosis, the fact remains that all these effects can have a very significant impact on the survival of amphibian populations.

# Toxaphene in the marine ecosystem of the St. Lawrence – status of contamination, ecotoxicology and human health

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Toxaphene is an organochlorine pesticide that was used intensively during the 1970s. In the early 80s, recognition of its toxicity led to its being banned in Canada and the United States. Despite these measures, significant quantities of this contaminant are found in the colder regions of Canada. In eastern Canada, very little data exists for the St. Lawrence marine ecosystem (SLME) on contamination by toxaphene. Two studies on beluga whales in the St. Lawrence Estuary show that these mammals are among the most contaminated on the planet. Because little is known about the presence and toxicological effects of toxaphene in the SLME, it is important to establish the extent of toxaphene dispersion in the SLME and assess toxicological risks to aquatic organisms and humans posed by the presence of this contaminant. This research provides new information on the distribution of toxaphene in the EMSL and on its toxicity to the human immune system and marine fish embryos.

To understand dispersion of toxaphene in the SLME, we studied 13 marine organisms that are representative of the food chain. They all contain toxaphene at concentrations below total PCB or DDT content. Worms living in sediments are the least contaminated, while seals and belugas have the high levels of contaminants. However, in fish intended for human consumption (herring and halibut), measured concentrations are below the recommended international limits. Our work suggests that the fish of the SLME meet the standards for human consumption. However, toxicity to aquatic organisms remains to be determined.

To study the toxicologic hazards associated with an exposure to toxaphene in fish, we looked at the possible relationship between spinal deformities in Atlantic tomcod captured in the SLME and toxaphene concentration in the livers of these fish. Our work shows that toxaphene concentrations are not are not related to the presence of spinal deformities in adult fish (3 to 5 years of age). Moreover, the prevalence of deformities does not increase with the age of the fish. Work is currently under way to test the theory that an exposure to toxaphene in the embryonic stage could play a critical role in the development of deformities. We recently induced spinal deformities in the embryos of a saltwater fish, the mummichog, exposed to commercial toxaphene. Future work will examine the embryotoxicity of toxaphene extracted from tomcod tissue samples, and try to establish the tissular concentrations that are associated with toxicity responses.

The study of toxaphene toxicity in humans is difficult because subjects cannot be deliberately exposed to this contaminant. In order to circumvent this problem, we have conducted in vitro toxicity tests on neutrophil cells, a type of white blood cell, isolated from human blood. These cells are part of the body's first line of defence against infectious agents. Our work shows that the effect of toxaphene on the behaviour of neutrophil cells is directly related to the concentration added. This suggests that the human immune system could be vulnerable to this contaminant. The mechanisms involved in the immune system response are currently being studied.

# Bioaccumulation of phthalate esters in aquatic food-webs

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Phthalates esters are industrial chemicals. They are used as plasticizers in polyvinyl chloride (PVC), polyvinylacetates, lubricating oils, automobile parts, paints, glue, insect repellents, photographic films, perfumes, and food packaging and many other products. Between 1980 and 1990, Canada and the US produced approximately 200,000 tonnes/year. The current global production level is approximately 4,300,000 tonnes/year. The substances are on the Priority Substances List and are considered to be chemicals of concern in Canada because they are oily substances that do not break down easily. The chemicals have properties that may cause them to accumulate in fatty tissues of organisms and biomagnify in the food chain. This may result in high levels in organisms at the top of the food chain. The revised Canadian Environmental Protection Act aims to identify substances that are bioaccumulative in a hazard and risk assessment. Substances that are persistent or bioaccumulative and toxic may be removed from Canadian commerce.

The objective of our study was to determine the ability of phthalate esters to bioaccumulate and biomagnify in a marine aquatic food chain. To accomplish this we sampled water, sediments and a range of organisms representing different types of aquatic food chains from 3 stations in a section of Burrard Inlet. We analyzed our samples and determined the concentrations of a range of phthalate esters in water, sediment and biota samples. To achieve this we needed to develop novel methods of analysis and detection to overcome background contamination problems and to be able to quantify the complex industrial mixtures of phthalate esters that are produced and used in commercial products. We found that concentrations of phthalate esters in the biological organisms sampled were many times greater than those in the water. This indicates that phthalate esters can bioaccumulate. The bioaccumulation factors of certain phthalate esters exceeded the bioaccumulation criterion of 5,000 in the Canadian Environmental Protection Act. We also found that phthalate esters do not biomagnify in the food chain. For certain phthalate esters we found concentrations in organisms at the top of the food chain to be lower than in organisms at the bottom of the food chain. This is sometimes referred to as trophic dilution, the opposite of biomagnification. The results suggest that phthalate esters are efficiently degraded when they are absorbed through the diet. However, when absorbed via the gills of the organisms the breakdown is less efficient and accumulation takes place. The significance of our findings is that phthalate esters are less bioaccumulative than PCBs. If the intent of the regulations under the 1999 Canadian Environmental Protection Act is to identify biomagnifying chemicals such as PCBs to be "bioaccumulative", then the results of this study indicate that phthalate esters are not "bioaccumulative" substances. This is important because if phthalate esters are not considered to be bioaccumulative or persistent, phthalate esters may not to be further assessed on their toxicological effects in the environment.

# Occurrence, fate, and effects of fluorinated surfactants in the Canadian environment

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Perfluorinated surfactants (FSs) are recalcitrant and persistent chemicals with wide-usage in industrial and consumer applications. While almost nothing was published about this class of chemicals when we started this project, we hypothesized they would be highly persistent and bioaccumulative, be widespread in the environment, and have the potential for toxicological effects. One year into our study the major manufacturer withdrew the entire product line due to their reaching similar conclusions. Our purpose in the research was to develop methods to carry out experiments with FSs, measure their environmentally relevant physical properties, determine their toxicological potential to aquatic plants and animals, and monitor their occurrence in the Canadian environment.

The approach we took was integrated across an interdisciplinary scientific team and included structural analogues to ensure that the knowledge learned was readily transferable to related classes of chemicals. We developed a suite of chromatographic and spectroscopic analytical methods to determine targeted FSs in water, air, biota, and soil and have applied them throughout the project. The primary method utilizes liquid chromatography coupled to a mass spectrometry system that gives us extremely sensitive and precise data although other methods including gas chromatography and fluorine NMR have proved essential to addressing certain knowledge gaps. We measured the physical properties of bioconcentration/bioaccumulation, sorption to sediment, vapour pressure, and Henry's Law constant for a series of perfluorinated carboxylates/sulfonates. Specific insight was obtained for the influence 'perfluorination' has on these physical properties. We learned that despite the full negative charge on the carboxylates/sulfonates that as the perfluorochain lengthens the propensity to bioconcentrate into fish and bind to sediment also goes up; each additional $CF_2$ group increases bioconcentration by a factor of 10. Vapour pressure determinations showed the perfluoro-tail of these molecules imparted surprisingly high volatility to these and related perfluorinated chemicals. Degradation studies have proven that the perfluorocarboxylates and sulfonates are inert even under extreme conditions of oxidation or reduction. Both lab and field aquatic toxicology studies, where full assemblages of aquatic plants and animals were exposed to individual and FSs mixtures, indicated that toxicity was generally low (ppm) but varied with chain length and polar functionality. Field monitoring has shown widespread occurrence of these chemicals in aquatic animals (ng/g), surface waters (ppq-ppt), and air (10-100 $pg/m^3$) and values 10 to 100x higher in contaminated areas which approached the adverse effects level. Our field measurements of biota showed the unexpected presence of longer chain perfluorocarboxylates and higher bioaccumulation factors than laboratory values. This may indicate a wider source for these materials than was previously realized. Identification and quantification of PFOS-based neutral alcohols in air samples supports our theory that these surface applied materials are likely responsible for the worldwide dissemination of PFOS.

The research is important because perfluorination results in unique properties that contribute to the fate, disposition, persistence, and toxicity and our project has made fundamental discoveries in each of these areas. FSs represent a new class of 'persistent organic pollutant' and indeed do so by redefining environmental persistence.

# Modelling the sustainable use of organic chemicals in a healthy continental environment

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The general objective of this project is to improve our ability to understand and predict how chemicals, including pesticides, which are used in distant locations such as Mexico or Alabama are transported to Canada where they may contaminate aquatic and terrestrial ecosystems. Computer programs, or mass balance models, are written to attempt to predict how such chemicals will behave. Such models have been widely and successfully used in the past to assess how chemicals partition between environmental media such as air, water, soils, sediments, vegetation and fish, where and how fast they are likely to degrade, how far they will travel, and ultimately what their concentrations will be in the various media of the ecosystem, including foodstuffs. The underlying philosophy is that since these chemicals can become widely dispersed in the environment, effective control is only possible if international coordinated efforts are made to reduce emissions.

Focussing on North America and persistent chemicals which are likely to be transported to Canada, Trent, in collaboration with the University of California at Berkeley, developed a continental contaminant fate model. Data such as area, soil type, and meteorology were gathered into Geographic Information System (GIS) format for the 24 ecological regions, primarily defined by watersheds. The first compound examined in this study was toxaphene; other chemicals of concern will follow.

Toxaphene is a persistent organochlorine pesticide that has been used on cotton crops in the southern United States, but is detected in the Great Lakes. The yearly emissions of toxaphene were estimated for each of the model regions between 1945 and 2000. In high usage regions of the southern United States there is satisfactory agreement between the model results and reported concentrations in air and soils. Around the Great Lakes there is good agreement between the model results and reported concentrations.

Two complementary models were developed. First is a model to rank chemicals based on their persistence and long range transport potential; key properties being examined by regulators when chemicals are chosen for restrictions. Second is a model of bioaccumulation in living things and foodwebs. It uses concentrations in the abiotic media of air, water, soil and sediment to calculate levels in vegetation, sediment-dwellers, fish, mammals, birds and farm animals. The entire set of concentrations can then be used as input to a human exposure model which calculates the amount of chemical per day that a person is likely to experience from each environmental medium, and each to chemical emission in any of the modelled regions.

Together these models provide a basis for predicting how chemicals will behave in North America, for understanding data from monitoring programs, and for identifying chemicals of greatest concern.

# Follow-up of preschool aged children exposed to PCBs and MeHg through fish consumption

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The effects of prenatal exposure to polychlorinated biphenyls (PCBs) and methylmercury (MeHg) resulting from fish consumption were studied in fish eating populations from various countries. Prenatal exposure to PCBs and MeHg has been linked to deficits in neurobehavioral development during infancy. However, only two studies provided data for school age children, and effects on neuromotor as well as neurophysiology were poorly documented. Although tests targeting neurophysiology were performed in one of these studies with school aged children exposed to MeHg, the study remained incomplete. Furthermore, no study has been aimed at investigating cognitive deficits under MeHg exposure using event-related potentials (electrical response from the brain to cognitive events).

The aim of this project is to examine the neuromotor and neurophysiological consequences at preschool age of prenatal exposure to environmental contaminants such as PCBs and mercury. The proposed project is designed to extend previous findings by studying long term effects and domains of effects that have been overlooked in most of the previous studies. Of particular interest is the impact of exposure on neurophysiological and neurological endpoints that could be related to learning difficulties and disabilities. Children of preschool age from Nunavik represent the target group. This study will not only clarify the impact of environmental contaminants on children's cognitive and learning abilities, but also provide current information about visual, hearing and neurological problems seen in children from Nunavik.

The sample comprised 103 Nunavik Inuit children aged between five and six years. Prenatal exposure to PCBs and MeHg was documented from cord blood analysis at birth. Groups will be comparable regarding the socio-economic status.

A neurological exam was performed, the fine and gross neuromotor function as well as the visual system and the cognitive and attentional functions were assessed. Demographic, socio-familial and biological potential confounds were assessed via maternal interviews and blood sample analysis. The electrophysiological results (cognitive and visual evoked potentials) of about 35% of children had to be discarded because of physical and cultural difficulties (e.g., hearing impairments, lack of attention during testing, difficulties understanding testing instructions).

Because reduction of data is currently underway, there are no results to present at this stage in relation to the study objectives.

# Sources, long range transport and impacts of new and old POPs inferred from dated sediment cores and lake waters

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The purpose of this study was to determine present and past inputs of persistent organic pollutants (POPs) such as PCBs, as well as possible new POPs, to Canadian freshwater systems by analysing dated sediment cores and lake surface waters. By collecting sediment cores over a wide geographic area we have been able to examine latitudinal and longitudinal trends in deposition and patterns of individual POPs. This information can be used to refine and calibrate long range transport and deposition models of POPs and to help priorize which new POPs are of greatest concern in terms of exposure of aquatic life in North America.

Sediment cores were collected from 28 lakes in southern and northern Canada as well as in north-eastern USA. A north-south transect centered on about 75° W longitude and an east-west transect at 45-50 °N latitude have been developed. Cores were collected in a deep zone of each lake where deposited material is likely to collect and sliced at 0.5 or 1 cm thickness. Only those with consistent sedimentation rates, based on radiochemical analysis using $^{210}$Pb analysis, and reasonably large mass per slice were being analysed for POPs. Large volume (19L) samples of lake surface (1 m depth) water collected from 30 lakes (1999-2001) including all of those that were cored - but including those where a core could not be collected or was undateable.

Sediment cores were analysed for POPs including old, now banned, organochlorine pesticides (e.g. DDT, toxaphene) and industrial compounds (PCBs, chlorobenzenes) as well as currently used flame retardants (e.g. brominated flame retardants and short- and medium chain chlorinated paraffins). Water samples were extracted by used of a resin column under clean room conditions to avoid contamination from POPs in building air. Water was analysed for current use chlorinated and organophosphate pesticides (e.g. endosulfan, disulfoton) and triazine herbicides (e.g. atrazine) using gas chromatography-mass spectrometry. Additional water samples were analysed for haloacetic acids (HAAs) and perfluorocarboxylate surfactants (PFCS). Water chemistry (pH, dissolved organic carbon, total Phosphorus etc) analyses were also conducted.

Major current use pesticides in lake waters were atrazine, metolachlor, alachlor, dacthal and disulfoton. Although atrazine and metolachlor use is restricted to corn growing areas, they both were detectable at low ng/L concentrations in remote lakes in non agricultural areas (NW Ont, NW Que, NW New Brunswick, Labrador) and at much higher levels in lakes in or near agricultural areas (Simcoe, Cayuga, Seneca).

Trifluoroacetic acid (TFA), a highly persistent end product of the degradation of HCFC refridgerants in the atmosphere (among other sources) was detectable in all lake waters at ng/L levels. TFA concentrations showed a latitudinal gradient with higher levels in lakes in Ontario than in northern Saskatchewan or the High Arctic. PFCSs were detectable at low ng/L concentrations in several remote lakes (e.g. in Adirondack Park and in northern Manitoba) indicating that these chemicals may be more widely dispersed than previously thought.

Deposition of PCBs and DDT-related compounds in sediment cores was highest in lakes in Ontario and New York state and declined significantly with latitude. Deposition rates of these chemicals were about 25-times lower at 80$^{o}$N in the Canadian high arctic than at 45$^{o}$N in southern Canada. Brominated flame retardants were detected in both arctic and mid-latitude cores although deposition was as much as 1000- times higher in the south.

These results confirm previous studies of PCB and DDT deposition to remote lakes in Canada and have identified, for the first time, several new persistent and bioaccumulative chemical contaminants in the same lakes in both sediments and water. The information will help assess which new chemicals may pose a risk to Canadian aquatic environments and which might be possible candidates for future listing as POPs.

# Biomagnification of persistent organic pollutants and mercury in Canadian freshwater subsistence fisheries and food webs

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The purpose of this project is to examine levels of persistent bioaccumulative toxic substances such as PCBs, DDT, toxaphene and mercury in top predator fishes and their food webs from lakes across northern Canada from northern Alberta to Labrador. Many of these lakes have important aboriginal subsistence fisheries We have selected this geographic area because it is currently understudied relative to the Great Lakes and the Arctic (north of 60°N) where numerous measurements have already been made on persistent organic pollutants (POPs) and Hg. The project involves collection and analysis of fish and other food chain organisms from lakes in northern Saskatchewan, north-western Ontario, central Ontario, Labrador, and central New Brunswick. Sampling is coordinated by members of the project team in each region working with local communities and provincial/federal fisheries officers. A total of 36 lakes are being studied over a three-year period. Twenty-nine of the lakes have lake trout as the top predator while brook trout, walleye, and pike are top predators in 8 lakes. In 18 of these lakes we are examining the transfer of food chain transfer of POPs by collecting of forage fish (yellow perch, lake herring, cisco, lake whitefish, smelt), zooplankton, amphipods (mysis), and benthic invertebrates. We are examining factors that can be used to predict lakes with elevated fish contaminant levels such as food chain length, dietary habits, growth and feeding rates, size spectrum and nutrient status of microplankton, lake trophic status, and the presence/absence of certain planktonic and benthic invertebrates.

Approximately 600 fish and invertebrate samples will have been analysed for PCBs, and organochlorine pesticides by the time the project is completed. DDT-related compounds (sDDT) along with PCBs and toxaphene are the major organochlorines in whole fish samples. Mean sDDT levels in lake trout ranged from 4 to 1410 ng/g wet weight (ww) and were generally highest in more southerly lakes, although Wabush lake in Labrador had unexpectedly high levels. PCBs were highest in lake trout from lakes in southern Ontario/upper NY State and concentrations showed a significant relationship with latitude.

In addition to the persistent organochlorine chemicals, brominated diphenyl ethers (widely used as flame retardants) are being determined in a subset of lakes. Pesticides, endosulfan and lindane, for which there are concerns about possible environmental persistence and toxicity, were found to range from <0.1-0.8 ng/g ww and <0.1-0.6 ng/g ww, respectively. Highest concentrations of endosulfan were found in trout in eastern Canada, especially from lakes in Labrador, while highest lindane levels were in fish from northern Saskatchewan. This agreed with known use patterns; endosulfan use is heaviest in eastern Canada while lindane is used primarily in Saskatchewan.

Mercury has been determined in 700 fish muscle samples, in the same fish that were analysed for POPs as well as in additional samples. Mercury levels ranged from 0.13 to 1.3 ug/g ww and were significantly different among lakes. About 10% of the fish sampled, ex-

ceeded the Canadian guideline, of 0.5 ug/g wet weight, for fish consumption. Within lakes, mercury concentrations were highest in larger, older individuals. Mean mercury levels (length adjusted) were significantly higher in fish (lake trout, walleye and brook trout) in eastern Canadian lakes and were inversely related to longitude. No relationship was found between adjusted-mercury concentrations and lake size, latitude, water chemistry or lake depth.

This study has provided baseline information on concentrations of POPs and mercury in fish from areas that are relatively understudied in terms of contaminant levels. It represents one of the largest datasets on fish contaminants from a single study. Results will be made available on a web site during 2002. The results show that POPs continue to be contaminants of concern in top predator fishes from Canadian lakes outside of the Great Lakes. Past local sources (DDT) may be more important than previously thought. Some of the among lake variation in POPs and mercury can be explained by distance from sources; for example, declining concentrations of sDDT and PCBs from south to north trend in sDDT, and increasing mercury from west to east.

# Effects of in utero exposure to POPs on reproduction and development

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Persistent organic pollutants (POPs) are stable, highly fat soluble, compounds that accumulate in the environment and in the food chain. Organochlorine food and environmental contaminants have been implicated in impaired fertility in humans, wildlife species and laboratory animal models. While many POPs, such as organochlorines, have been banned in Canada since the 1980s, they remain present at high concentrations in several areas. Inhabitants of the Arctic are exposed to high levels of organochlorines in their diet. We hypothesized that in utero exposure to a mixture of organochlorines and polychlorinated biphenols, which have been identified as components of the diet of Inuits living on Broughton Island, would have adverse effects on gonadal and reproductive tract development.

In determining the consequences of exposure to POPs on fetal development, as well as gonadal, reproductive tract and thyroid functions, groups of adult female SD rats were treated either from days 0-20 or 8-20 of gestation with POPs (n=12/group). For each of these two windows of exposure, four groups of pregnant females were gavaged with vehicle, or 10, 100 or 1000 times the doses that reflect exposure to dietary POPs. Using this protocol, two experiments were done: 1) females were euthanised on day 20 of gestation and pregnancy outcome, histological appearance of gonads, the gene expression profiles of maternal and fetal liver and gonads, and thyroid development were assessed; 2) the mothers were allowed to deliver normally and the postnatal development of the pups to adulthood was monitored, at which time a systematic study of general markers of toxicity as well as selected biomarkers in key organ systems was done. The levels of individual components of the POPs mixture were found to be 10 to 500-fold above those in controls in maternal livers of treated animals on day 20 of gestation. There was no effect of POPs exposure on numbers of pregnant females, pre- or post-implantation loss, fetal weights, sex ratio, or on the incidence of structural malformations. Histological assessment of fetal ovaries on day 20 of gestation revealed a reduction in the number of oocytes and an increase in apoptotic cell death; the ratio of Sertoli to germ cell number increased significantly in the testis. Furthermore, electron microscopic analysis revealed that the nuclear envelope of germ cells was highly convoluted and scalloped in high dose treated animals.

RNA was isolated from maternal and fetal livers and gonads and used for gene expression profiling. The main effect of the accumulation of POPs was a decrease in the numbers of genes expressed in maternal liver; such changes may result from inhibition of transcription or decreased transcript stability. There was little effect on gene expression in fetal livers. Several genes in the cyclin family were reduced in fetal ovaries but increased in fetal testes; key steroidogenic enzyme genes were reduced in fetal testes; these genes were expressed at very low level in fetal ovaries.

POPs doses which did not cause maternal toxicity induced growth retardation in both male and female pups before weaning and in females after weaning. In utero exposure to POPs resulted in a delay of developmental markers in both male and female pups, had no effect on the age of vaginal opening, but resulted in a significant delay in the age at first estrus, suggesting that POPs do play a role as endocrine disruptors.

These data provide evidence that in utero exposure to a mixture of POPs found in the diet lead to adverse effects on maternal hepatic gene expression, gonadal development, and postnatal development in rats. Insight into the mechanisms by which mixtures of POPs act as endocrine disruptors will give us novel approaches to human risk assessment.

# Endocrine-disrupting effects of persistent organic pollutants in free-ranging Pacific killer whales

Peter S. Ross

The high position of killer whales in the ocean's food chain makes them vulnerable to some of the pollutants that society has produced over the past 60 years. Of particular concern are the chemicals referred to as "Persistent Organic Pollutants" (or POPs), a catch-all class that includes many oily chemicals that do not easily breakdown in the environment. POPs include the polychlorinated biphenyls (PCBs), dioxins, and numerous pesticides like DDT. Despite many of these chemicals being banned in Canada, concerns linger: their persistence, continued leakage from landfill sites in Canada, continued usage in other parts of the world, and chemical characteristics mean that wildlife are still being exposed to worrisome concentrations. In some areas, the health of wildlife has been affected, with the reproductive, endocrine and immune systems being particularly vulnerable to the effects of these chemicals.

We have been studying the possible effects of high POPs levels in Pacific killer whales by measuring several "health parameters" in very small dart samples of skin and blubber. As part of our research, we recently found that Pacific killer whales are highly contaminated with PCBs. Since the chemicals that we are concerned with are fat-soluble, we can easily measure their concentrations in the blubber. In addition, we have developed several ways to measure the "health" of killer whales by measuring enzyme and hormone levels in both the blubber and the skin fractions of our samples. Because both natural and anthropogenic (chemical) factors can affect the health of wild animals, an important feature of our work has been to document the effects of age, sex, diet and condition on our "health" measurements.

We also collected samples of several other species, representing some of the things (fish, marine mammals) that killer whales eat. By measuring the POPs in these prey items, we will better understand where these chemicals in the killer whales are coming from. We have found that some chemicals are easily broken down and eliminated by killer whales, whereas others are not. Such work helps us to understand which chemicals may be the biggest threat to the health of killer whales.

Finally, we have developed a model to predict the concentrations of different chemicals in British Columbia's killer whales at any point in time (past or future). This provides managers in government with a powerful tool to understand how quickly things might improve for killer whales if we stop using a certain chemical. By occupying the top of the food chain in the Northeast Pacific Ocean, killer whales are serving as "canaries of the ocean" and are pinpointing which chemicals may present a health risk to wildlife and certain human consumers.

# Assessment of potential effects from contaminant exposure on beluga whales (*delphinapterus leucas*) and polar bears (*ursus maritimus*): reproductive and endocrine systems

Sang Susan, Michael Kwan, Muir Derek, Daniel Leclair, Mikaelian Igor

Over the past decade, Inuit hunters, trappers and fishers have reported a wide range of abnormalities in their harvests. The cause of many of these abnormalities is unknown. However, the perception in Arctic communities is that environmental contaminants – persistent organic pollutants (POPs), heavy metals and radionuclides – may be responsible for these anomalies. The difficulty is in relating the observed abnormalities to these environmental contaminants.

In 1999, World Wildlife Fund Canada (WWF) and the Nunavik Research Centre (NvRC) began a community-based sampling and monitoring project of beluga whales and polar bears in Nunavik. The objective of the project is to increase the understanding of the relationship between exposure to contaminants and wildlife health effects. The project is designed to investigate the concentration of persistent organic pollutants (POPs) and heavy metals, as well as, examining the parasitic infections and histological lesions in reproductive tract and endocrine organs of beluga whales and polar bears harvested in four Nunavik communities. Due to difficulties in obtaining polar bear samples, the study is now focused only on harvested beluga whales.

In the first year of our study (1999-2000), we collected tissue and organ samples from 15 beluga whales for contaminants analysis and pathological and histological examinations.

The results from contaminant analysis confirmed that PCBs were the major organochlorine present in the whale blubber followed by DDT and chlordane-related compounds. Higher concentrations of alSium were also found in kidneys. Lead and arsenic levels were generally low in all tissues. Selenium was found to be highest in the skin and liver samples.

The whales were also examined for parasitic infection and their thyroid and gonads were looked at for histological abnormalities. Field observations and microscopic evaluations showed that most whales harvested were healthy, however, some peculiarities were also noted which suggest further research. Examples are presence of possible parasitic infection in three beluga whales, absence of eggs in the ovary of one whale, and presence of suspected parasitic cysts affecting the genital system in four whales (one female and three males).

# Assessment of neurotoxic effects in a First Nation community exposed to PCBs

Harold Schwartz

The traditional lifestyle of First Nations includes a high consumption of country foods such as fish and wild game. While theses dietary habits are known to be intrinsically healthy, they may put First Nations at higher risk of exposure to polychlorinated biphenyls (PCBs), DDE, methylmercury and other contaminants when their environment is contaminated. The objective of the present study was to assess whether neurobehavioral deficits were associated with PCB exposure in an Indigenous community of the Sioux Lookout Zone, northern Ontario. Since other contaminants may be present in association with PCBs, we also assessed the possible effects of p,p'-DDE and mercury and explored possible additive relations with PCBs. Participants were recruited from a list of 101 names randomly selected from a Health Canada exposure assessment study carried out in 1999 in the same community. A total of 54 persons (53.5%) from this group returned for testing; a further 45 persons were recruited from the community with similar socio-demographics to those that had not returned. The final study group included 50 men and 49 women. Participants provided blood and hair samples, underwent testing of cognitive, motor and sensory functions and responded to questionnaires including a dietary survey, socio-demographic information, lifestyle, medical history, health symptoms and quality of life. Blood samples were analysed for 8 PCB congeners, Aroclor 1254 and 1260, as well as p,p'-DDE, and hair samples were analysed for total mercury (Hg) at the First Nations and Inuit Health Branch Laboratory (Health Canada). Statistical analyses were based on different analytical multivariate strategies to overcome the effects of confounding variables, such as age and education, which are strongly correlated to each other, to the bioindicators of exposure and to performance on the test battery. Results showed that those with higher levels of PCBs for their age performed significantly more poorly on cognitive tests which assess visuospatial planning and organisation. Those in the upper 75$^{th}$ percentile of exposure to Aroclor 1260 for their age category were 5 times more likely to be in the lowest 25$^{th}$ percentile for their age category on a test which requires copying a complex figure: OR = 4.99 [1.72 – 14.52]. The older persons showed the highest deficits. On a test of visual attention and reaction time those in the higher Aroclor 1260 category were 3 times more likely to be in the lowest 25$^{th}$ percentile for performance for their age category: OR: 3.00 [1.03 – 8.76]. Persons in the upper 75$^{th}$ percentile score for their age likewise were 4-5 times more likely to be in the slowest group on the manual dexterity, but not on the other motor tests or the sensory tests. Serum p,p' DDE levels were likewise associated with poorer performance on many of the cognitive tests, as well as on some of the psychomotor tests. While hair Hg levels were associated with poorer performance on some of the psychomotor tests, exploratory analyses showed that the greatest deficits on certain tasks were observed among those with the higher levels of both PCBs and DDE. This was also the case for Hg and PCBs in relation to reaction time, suggesting that there may be additive effects. Diabetes was examined as a possible confounder and unexpected finding of this study was that DDE levels were significantly higher among those who report diabetes. Diabetes was positively associated with age, body mass index and serum DDE and negatively with the frequency of trout consumption, supporting the premise that fish consumption may be protective. The DDE-diabetes association does not tell us whether it is because DDE contributes to diabetes or because those with diabetes accumulate more DDE. These findings demonstrate that PCB exposure may affect cognitive functions, particularly in the visuospatial domain. There may be additive effects with DDE and/or mercury. Further studies should examine PCB exposure in other communities focus on possible additive or synergistic effects of multiple contaminants, as well as the interaction with diabetes, which is highly prevalent among First Nation communities.

# Multiple stressors effects on native amphibians

Dean Thompson, Barbara Wojtaszek, Andrea Edginton, Celia Chen, Gerry Stephenson, Herman Boermans, Brendan Hickie

Small wetlands of north-eastern North America are critical habitats for numerous amphibian species and susceptible to contamination by both forest-use herbicides and acid deposition. In this context, a collaborative project has been undertaken to examine potential interactive and multiple stress effects of two herbicide formulations - VISION (glyphoste) and RELEASE (triclopyr) on native amphibian species - northern leopard frog (*Rana pipiens*) and green frog (*Rana clamitans*), under environmentally relevant pH regimes. Tier I studies have focused on comparative sensitivity of different amphibian species and life stages using standardized laboratory testing protocols. Tier II studies examined particular interactions among herbicide, pH and food availability as potential stressors. Tier III studies utilized enclosures deployed in two different wetland ecosystems to examine impacts on caged amphibians as well as populations of phytoplankton and zooplankton under natural environmental conditions. Results of tier III studies will also be used as a basis for developing and validating multimedia chemical fate models suitable for predicting chemical fate in aquatic enclosures. Tier IV research involved chemical and biological monitoring of operational herbicide applications to quantify herbicide concentrations in a variety of natural wetlands within, adjacent to or buffered from forest regeneration sites receiving aerial herbicide treatments.

Results of tier I studies indicate that late larval stages are more sensitive than embryos and that the four amphibian species tested are generally equi-sensitive to the two herbicides tested. Both tier I and II studies demonstrate significant but differential interaction among herbicide and pH stressors, with high pH enhancing the toxic effects of VISION whereas low pH enhanced toxicity of the RELEASE formulation. Tier II studies also suggest that food deprivation stress further increased toxic effects. Overall, laboratory studies indicate a substantial potential for deleterious effects at environmentally relevant pH and herbicide concentration regimes. Field studies generally confirmed that herbicide contamination, pH, food stress and anoxia were relevant and concomitant stressors in forest wetland ecosystems. However, effects in field studies were moderated relative to effects which may have been predicted based on laboratory studies. This project demonstrates that multiple stressors occur concomitantly in natural wetlands associated with herbicide-treated forest regeneration sites and that these may be important determinants of overall effects on native amphibians. Results suggest that both laboratory and field studies are required to fully characterize environmental risk associated with anthropogenic stressors in natural environments.

# Development and field testing of a passive air sampling technique for persistent organic pollutants

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In order to investigate large scale patterns of persistent organic pollutants (POPs) in the North American atmosphere, we are developing an air sampling technique, which is based on the passive uptake of the contaminants in a synthetic sorbent resin. The amount of POPs accumulated in a passive sampler will steadily increase until saturation is approached. If the uptake capacity of the sampler is sufficiently large, averaged air concentration can be derived from the accumulated amount and knowledge of the sampling rate. The advantage of passive air samplers over existing sampling techniques is that they are cheap, simple and do not require electricity or skilled labour for operation.

In laboratory experiments, we determined both the uptake capacity and the uptake rates of the passive samplers we are designing. We confirmed that the sorption coefficients of the more volatile POPs onto the sampler material at ambient temperatures are so large that the passive samplers do not approach saturation even after very long deployment periods in the range of years. In wind tunnel experiments, information on the rate of contaminant uptake was obtained and these rates were found to be independent of wind speed.

Field testing of this technique involved the determination of the uptake kinetics of various POPs, including polychlorinated biphenyls and organochlorine pesticides, over an entire year at three calibration stations with ongoing active high volume sampling. Located in the Great Lakes region and the Canadian High Arctic, these stations span a wide range of meteorological conditions and air contamination. The field samplers showed levels above blank values within a few weeks of deployment. Levels continued to increase linearly with increasing length of field exposure. Reasonable agreement between duplicate samplers was observed. With the help of the wind tunnel derived sampling rates, the amounts accumulated in the field samplers were converted into volumetric air concentration. These agreed well with the levels measured using the classical active sampling technique.

Due to its simplicity and low price, this technique allows monitoring networks involving a large number of sites, including remote locations. They should thus be particularly suitable for deployment in the Arctic and in high mountain regions, as well as in developing countries. The feasibility of such a network is being tested in North America, where more than a hundred of these samplers have been deployed for an entire year at numerous locations in Canada, the U.S.A., Mexico, Belize and Costa Rica. Two transects in the North-South (Ellesmere Island to Central America) and East-West (Eastern Newfoundland to Western Vancouver Island) direction span 72 degrees of latitude and longitude. A third transects covers 3000 m of altitude from the Pacific Ocean to the Rocky Mountains.

# Metals

Metals are naturally occurring substances, some of which have been linked to adverse effects on human health and wildlife. Since its launch in 1998, the TSRI has allocated over $6 million in funding 18 research projects in the area of Metals.

In fiscal years 1999-2000 and 2000-2001, applicants were asked to accelerate research activities designed to understand exposure to and sources, loadings and the toxicological significance of specific forms of metals in the environment.

### The priority knowledge needs in fiscal year 1999-2000 were:

- To focus on priority metal forms (species of mercury, lead, cadmium) and environmental mixtures that may affect their toxicity;
- To determine the contribution of both natural and anthropogenic sources of specific metal species to environmental levels, as well as the impacts of global and regional changes in these sources;
- To determine loadings, valency states and rates of transformation to bioavailable forms for priority metals in the environment;
- To determine the toxicological and ecological impact of bioavailable forms of metals in the environment;
- To study the effects of acidification on the release of toxic metal species into the environment; and
- To develop methods for evaluating the toxic effects of consuming country foods in relation to their social, nutritional and economic benefits.

### In fiscal year 2000-2001, the knowledge needs were:

- To develop new approaches to assess the toxicological significance on human health and the environment of low level exposure to metals in food chains with an emphasis on at-risk populations such as the consumers of country foods; and
- To determine the contribution of both natural and anthropogenic sources of specific metal species to environmental levels, as well as the impacts of global and regional changes in these sources.

### In fiscal year 2001-2002:

There were no call for proposals in fiscal year 2001-2002, therefore, no knowledge needs were developed for any of the priority research areas. However, there were still ongoing research projects which were previously approved in the 1999-2000 fiscal year.

# Study of the physical and chemical evolution of aerosols in smelter and power plant plumes

Catharine Banic, Sam Daggupaty, W. Richard Leaitch, Henry Wong, Iain Campbell, Zdenek Nejedly, Jim Skeaff, Dogan Paktunc, Kevin Strawbridge, Clément Gariepy, Amares Chatt, Marc Lamoureux, J. Ian MacPherson, Hélène Gaonac'h, Diane Michelangeli

A study of particles and mercury emitted to the atmosphere from specific sources was undertaken by Environment Canada, the National Research Council of Canada, Natural Resources Canada, the University of Guelph, University of Quebec, Saint Mary's University, Dalhousie University, McGill University, York University and the Ontario Ministry of the Environment in partnership with the industries involved, Ontario Power Generation, Inc. and Noranda, Inc.

The National Research Council of Canada Twin Otter aircraft was flown in the plumes of the Nanticoke coal-fired power plant and the Horne copper smelter to investigate the sizes and metals content of the particles and the speciation of gaseous mercury present in the plumes. Knowledge of these properties is critical to the determination of the deposition rates of the metals emitted. The results obtained are being used to specify the properties of particles in existing atmospheric models.

Forty-seven flights were made into the plumes to characterize the emissions as they evolved under winter and summer conditions. A typical flight involved tracking the plume out to 30 km. The plumes were observed to contribute to the particle volume at particle diameters from 0.03 to 30 µm. Results of a 3-dimensional modelling study performed for selected time periods for winter conditions show that the deposition to the surface within 100 km of the sources of particles in the size range 0.25 to 2 µm is, insignificant and for particles with diameters between 16 and 24 µm is up to 12%. Since the smaller particles are not lost by dry deposition they will have lifetimes in the atmosphere until removal by in-cloud processes, which may take up to two weeks. Current data analysis concentrates on specifying the metal content of the particles in the plumes as a function of particle size. For example, under the conditions analyzed to date, the smelter emissions showed at least 50% of the lead emitted to be associated with particles smaller than 2 µm. When this analysis is done for a number of metals and for both sources, the atmospheric processing of the emissions will be modelled in a realistic way.

In addition to the aircraft measurements, a scanning lidar system was deployed at the ground to monitor the behaviour of the plumes. Remote sensing (time series and plume transects) of the $SO_2$ in the plume was made at the copper smelter with a COSPEC spectrometer. Measurements were made at ground-based sites to characterize the chemical properties of the regional aerosol into which the plumes were emitted.

This study is part of the Canadian Metals in the Environment Research Network (MITE-RN) and was jointly funded by the Toxic Substances Research Initiative, the MITE-RN and the Meteorological Service of Canada. It has close ties with the MITE program undertaken by the Geological Survey of Canada (GSC). Together, these research projects generate coherent data sets that will be valuable in the international and domestic risk assessment of metals.

# How sediment influences amounts of metal accumulated by bivalves

Leah Bendell-Young, Margo M. Moore, Daryl Crozier, A. Jurgensen and J. King

We are currently developing and applying methodologies for X-ray absorption fine structure spectroscopy (XAFS) using the synchrotron radiation beamlines provided by the Pacific Northwest Consortium Collaborative Access Team (PNC-CAT). These methods are being used to geochemically characterize complex environmental matrices such as bacteria-manganese oxide composites and natural sediments.

Two separate but related objectives are being addressed: 1) The role of bacteria in mediating metal availability to the filter-feeding mussel, *Mytilus trossolus* and 2) The role of sediment geochemistry in influencing metal bioavailability to sediment ingesting organisms. In both studies methods, using XAFS have been developed and are being applied to more accurately identify the speciation of metals in complex sediment matrices with this speciation then linked to metal accumulation by sediment ingesting organisms.

So far we have successfully; 1) determined the optimal growth conditions of the bacteria *Leptothrix discophora* under several metal exposure regimes, 2) identified the structure of the manganese oxide casing precipitated on the surface of bacteria as birnessite 3) compared the accuracy of trace metal speciation in complex matrices through XAFS versus wet extraction methods; XAFS identified the main sorptive phase in natural sediments low in oxides of manganese 4) determined the role of bacteria in mediating lead availability to filter-feeding organisms. Our results so far suggest that manganese oxides that are formed in the presence of bacteria may actually reduce the amount of metal that a filter-feeding organism is exposed to via its diet.

Filter-feeding organisms such as mussels are key species in complex food-webs; they act as a conduit for the transfer of toxic trace metals from primary trophic levels to higher levels such as birds and humans. Therefore, it is extremely important to determine what factors, either biological or chemical, influence the accumulation of these metals by filter-feeding organisms. Our work has shown that one chemical factor, manganese oxides (an inorganic component of sediment) formed in the presence of bacteria may serve to reduce metal uptake by mussels. If true, then in aquatic environments where there are lots of oxides of manganese, metal uptake by filter-feeders may be less as compared to aquatic environments where oxides of manganese do not occur. Being able to predict the conditions where metal accumulation is greatest by organisms that occupy central positions within complex food chains will help us determine the conditions under which maximum trophic transfer of toxic metals occurs. This in turn will aid us in predicting where higher trophic levels, such as humans, are at greatest risk to the exposure to toxic trace metals through the food chain (i.e., from what we eat!!).

# The effects of mercury on oxidative status and neuromotor functions

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The primary goal of this study is to explore the effects of methylmercury (MeHg) on certain brain functions and on the markers of oxidative stress. The study will help us to better understand the toxicity of mercury, particularly with regard to neurological and cardio-vascular diseases. The study population consisted of two distinct groups. The first was made up of workers, employed by Hydro-Québec and based in Baie-James, who are sport fishers and consume large quantities of predatory fish (pike, pickerel). The exposure of these individuals is therefore limited in time. The second group consisted of Inuit adults recruited from the Nunavik community of Salluit. Because their diet consists primarily of fish and marine mammals, Inuit are particularly vulnerable to food-chain contaminants (organochlorines, heavy metals).

The initial data was collected in Baie-James in June 2000 before the start of the fishing season, and then again in November of the same year, after the fishing season ended. In this way, a total of 39 workers were seen on two occasions. In addition to participating in various tests to evaluate neuromotor functions (microtremors, postural balance, reaction time, hand-eye coordination in pointing tasks, rapid alternating movements, rate of hand movement), participants also supplied several biological samples to be used to measure mercury concentrations (hair, blood) and assess the various indicators of oxidative stress (blood, plasma and urine). Finally, participants were interviewed for information on their medical history, any medications they were taking, and certain lifestyle habits (consumption of drugs, alcohol and tobacco). The second round of data was collected in Salluit in February and March 2001, with 125 participants recruited. A total of 112 individuals completed the series of tests (same series as the one administered in Baie-James). Samples were also taken for selenium, PCB, pesticides and cannabis. Selenium is a nutrient that many researchers regard as an antitoxin against the effects of mercury. PCB, pesticides and cannabis samples were collected because, if present in elevated concentrations, these substances could compromise the results of the neuromotor tests. Sample analyses and neuromotor tests have now been completed, but results are not yet available. The final stage will involve combining all the available databases and carrying out more in-depth analyses aimed at achieving the goals of the study.

# The Mechanism of Cadmium and Lead Nephrotoxicity in Developing and Mature Kidneys

Sudhir D'Souza
University of Western Ontario

This study will use the mouse as a model to evaluate the mechanisms of toxicity of lead and cadmium on developing and mature kidneys, as well as examining the potential repair processes which may be involved in the regeneration of kidneys damaged by cadmium and lead exposure.

# The role of biogenic minerals (Fe-oxides) in trace metal sorption in aquatic environments

D. Fortin, F.G. Ferris and G.G. Leppard

The mobility of toxic metals in lakes is strongly affected by their immobilization onto the surface of mineral and biological particulates. Iron oxides represent a minor constituent of lake sediments but they are thought to play a major role in metal cycling. Their physical characteristics (such as small grain size, large surface area and poorly crystalline structure) make them efficient metal scavengers. Iron oxides present in lakes sediments can form as a result of pure chemical reactions but also in the presence of microorganisms (bacteria). Since bacteria are abundant in lakes, it is very likely that iron oxides will form on their surface, but the effect of bacteria on the formation and characteristics of the biogenic iron oxides is not well known. In addition, the role of biogenic iron oxides and bacteria in metal immobilization (sorption) is still under investigation.

The present project was designed to look into the role of various bacteria in the formation of biogenic iron oxides under conditions representing lake sediments and to determine the sorption capacity of those iron oxides for two toxic metals, Cd and Pb. The characterization of the biogenic iron oxides was based on bulk (mineral identification) and particle analysis, using electron microscopy. Metal sorption experiments were performed under laboratory conditions mimicking natural aquatic conditions and quantified with appropriate adsorption models. Results from our study showed that the presence of bacteria (4 different species) did not affect the mineralogical characteristics of the Fe-oxides. The oxides formed in the presence and in the absence of bacteria were identified as lepidocrocite, a common iron oxide found in lake sediments. The presence of bacteria did however affect the size of the particles and their crystalline order. In fact, biogenic oxides were smaller than the ones formed in the absence of bacteria and showed a poor structural order. The synthesis of the biogenic Fe-oxides was also performed in the presence of impurities commonly found in lakes (such as soluble silica, phosphate and sulfate), in order to assess their role in the formation of the oxides. The presence of silica in the various systems led to the formation of ferrihydrite, a common iron oxide found in sediments, whereas the presence of phosphate promoted the formation of small crystals of lepidocrocite similar to the ones formed in the presence of bacteria. Sulfate had no effect. Metal sorption results showed that the sorptive capacity for Cd was greater for the biogenic Fe-oxides and the individual cells than for the abiotic Fe-oxides. In addition, the sorptive capacity of abiotic Fe-oxides for Pb was greater than for Cd. The maximum sorption capacity for Pb for the abiotic Fe-oxides was however similar to the one reported for biogenic Fe-oxides, with the exception of the abiotic Fe-oxides formed in the presence of silica.

Our results suggest that the mobility of Pb and Cd is strongly influenced by the presence of bacteria and, in some cases, by the presence of biogenic Fe-oxides. Our findings also indicate that the role of bacteria in metal cycling should not be neglected, along with their role in mineral formation, especially for minerals susceptible to affect the mobility of toxic metals, such as Fe-oxides.

# Immunotoxicity of metals

J. Bernier, M. Fournier, E. Kouassi

Among environmental contaminants recognized for their toxicity and worldwide distribution, heavy metals are certainly of first concern. Studies in laboratory animals and epidemiologic data of human cohorts occupationally exposed to these chemicals indicate that metals can affect several physiological systems of the body. They can also elicit a number of immunomodulatory effects leading ultimately to an enhanced susceptibility to microbial agents and to the appearance of neoplastic diseases and autoimmune phenomena. Our group has contributed to demonstrate the immunotoxic potential of metals in several laboratory and wildlife species and elucidate mechanisms of toxicity.

Published data on the immunotoxic effects of toxic metals are often conducted at levels higher than environmental levels, or at concentrations higher than that found in human blood. Since humans are exposed to mixtures of toxic metals, the effects of mixed exposures require evaluation. Understanding of the effects of mercury (Hg), cadmium (Cd) and lead (Pb) on functions of human immune cells would help in the determination of risks associated with human exposure at physiologically relevant human blood levels of these metals. This would help in the design of strategies in preventing chronic Hg, Pb and Cd toxicity and deciding and setting acceptable safe environmental and human blood levels for those metals. A number of *in vitro* specific assay endpoints which measure humoral, cellular and nonspecific immune functions will be evaluated in human peripheral blood cells. Further, the project would help in determining whether immunotoxicological studies of Pb, Cd and Hg often conducted in mice and rats or mouse and rat cells are suitable models for humans, and if they are reasonable representations of effects of toxic metals on human immune cells.

The first objective of this research program was to establish the doses of metals, individually or in mixtures, that are killing human cells. To achieve this objective, dose-response experiments were performed. Only Hg was found cytotoxic at doses relevant to human contamination. We have also characterized the typical form of neutrophil cell death induced by high concentrations of Hg as being primary necrosis supervened over apoptosis. To further elucidate the biochemical signals involved, we studied the effects of necrogenic concentrations of Hg on intracellular events. The results showed that the cytotoxic effects were closely related to increases in cell calcium mobilization and to inhibition of mitochondrial membrane potential. Effects of metals on immune cells competence was also characterized using, once again, dose-response experiments. The effects were studied on the competence of lymphocytes, NK cells and neutrophils. When analysing the results, it appears clearly that mercury represents the most toxic metal, at human blood relevant doses. The effects of mercury exposure were greater on lymphocytes and NK than on neutrophils.

Mercury represents, therefore, a metal for which level of contamination present in human blood is quite similar to the level of toxicity.

# Solubility and Speciation of Trace Metals in Eastern Canadian Soils

William H. Hendershot, François Courchesne and Les Evans

Concern for trace metal contamination of soils is closely linked to fears that humans will be affected. There is also widespread concern that large scale contamination of ecosystems will lead to a general degradation of the environment. For example, the transport of trace metals such as Cd, Hg and Pb from contaminated soils to surface and ground waters is a crucial process regulating the quality of water for human consumption. A key process controlling the movement of Cd, Hg and Pb through soils, their leaching towards water bodies as well as their uptake by plants is the binding of trace metals by soil particles. This binding can render the contaminants relatively immobile and difficult for plants to absorb. Clearly, a systematic understanding of the processes that regulate the movement of trace metals is vital to insure the sustainability of terrestrial and aquatic ecosystems and to improve the predictive power of models used for management purposes.

The long term objective of this study is to quantify and model the binding mechanisms between trace metals (Cd, Hg and Pb) and the solid phase of soils, and with dissolved organic matter (DOM). The specific objectives are 1) to characterise the solid phase of soils, 2) to quantify the retention of Cd, Hg and Pb by soils under a range of environmental conditions (pH, metal and DOM contents), 3) to estimate the speciation of Cd, Pb and Hg in the solutions produced during the retention experiments, 4) to establish relationships between trace metal retention constants and commonly measured soil properties and, 5) to model the binding of Cd, Hg and Pb by soils using a surface complexation approach.

The sampling strategy encompasses soils that come from different biogeoclimatic regions in southern Ontario and Quebec and includes both mineral and organic soils. The trace metal contamination in these soils is due to mining and smelting of metals, other types of industrial activity such as battery recycling, or from railway maintenance yards. Agricultural fields contaminated from excessive sewage sludge application or pesticides containing Hg were also sampled. Approximately 30 soil samples were collected from sites with a wide range of chemical and physical properties. The use of such varied biogeoclimatic settings is essential for the generalisation of our findings.

Our preliminary results show that pH has a marked effect on the solubility and speciation of Cd and Pb and that Cd tends to exist mostly as free ion species in the soil solutions. With respect to metal availability to biological membranes, root elongation studies indicate that bioavailability is, in part, a function of metal speciation with free metals being more available. Moreover, the Cd and Pb binding constants in mineral horizons are close to the values reported in the literature, indicating the complexation model is applicable in a range of soil environments. In organic horizons, the data suggest that the differential uptake and accumulation of metals by trees play a central role in the post-depositional redistribution of Cd and Pb in the forest floor. Advances in the area of trace metal research will enable scientists to better predict the behaviour and fate of Cd, Hg and Pb in contaminated soils and contribute to the clarification of the role of trace metal speciation in the bioavailability of these metals to plants and soil organisms.

# A Chemical and Ecotoxicological Assessment of the Impact of Marine Tailings Disposal

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This study consists of a field examination at two historic mine sites in Newfoundland for the potential of mine tailings stored under salt water to leach trace metals such as arsenic, cadmium, copper, nickel, lead, and zinc. The research will lead to a better assessment of the risks and benefits associated with marine tailings disposal.

# Significance of arsenic speciation [As(V) vs. As(III)] in expression of arsenic toxicity in aquatic organisms: implications for Canadian water quality guidelines

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Arsenic contamination of aquatic ecosystems from anthropogenic sources such as mining (e.g., gold, uranium) is a concern in many areas of Canada. For example, arsenic concentrations in surface waters and sediments close to many northern Canadian mine sites, currently exceed Canadian water quality and draft sediment quality guidelines. In addition, these guidelines were set without due consideration of arsenic speciation (e.g., As(III), As(V)) which can influence toxicity. The specific chemical arsenic species that dominates in an aquatic environment depends on the physicochemical and biological characteristics of the water and sediment. One such characteristic is dissolved oxygen (DO) condition which when low, in addition to influencing arsenic speciation, may also act as a secondary stressor thereby potentially increasing arsenic toxicity.

This study aimed to determine the combined effect of arsenic, as either As(III) or As(V), and DO stress on juvenile stages of three benthic macroinvertebrate species with different habitat preferences and different sensitivities to both stressors. Experiments were performed in specially designed experimental systems where DO could be maintained constant over the 10-14 d test duration. The concentrations of both As(III) and As(V) were measured during toxicity tests to determine actual arsenic exposures, and As transformation rates where applicable.

The mayfly, *Baetis tricaudatus,* was the least tolerant of low DO, whereas the midge, *Chironomus tentans*, was the most tolerant; the amphipod, *Hyalella azteca*, had intermediate sensitivity. In terms of invertebrate species sensitivity, *B. tricaudatus* was ten times more sensitive to As(V), and eight times more sensitive to As(III), than *C. tentans* under high DO conditions. In terms of As toxicity, As(III) was six times more acutely toxic to *C. tentans* than As(V). As(III) was also more acutely toxic to *B. tricaudatus*, but there was no difference in *B. tricaudatus* growth at sublethal exposures to both As(III) and As(V). There was not a clear difference in sublethal toxicity of As(III) and As(V) to *C. tentans* either. When DO condition was considered as an interacting factor, differences in arsenic toxicity were observed for *C. tentans* and *H. azteca*, but not for *B. tricaudatus*. For *C. tentans*, for example, exposure to As(III) under low DO conditions (1.5 mg/L) increased larval mortality by ~30% and decreased growth by 50% relative to high DO conditions (6.5 mg/L). Although animals were primarily exposed to As(III) in the As(III) experiments, some As (III) did convert (likely via microbial activity) to As(V), resulting in conservative estimates of As(III) toxicity.

This study was one of only a few that has considered actual speciation when determining arsenic toxicity to benthic macroinvertebrates. The results indicate that arsenic speciation can be important and should be considered in the design and interpretation of arsenic toxicity tests and arsenic environmental risk assessments.

Furthermore, low DO levels, which are often associated with effluents and sediments, can enhance arsenic toxicity by both influencing arsenic speciation conversion rates and by placing animals under additional metabolic stress. Developing a better understanding of such interactive effects between arsenic and DO could influence a number of federal programs (e.g., the national EEM metal mining program, national dissolved oxygen and arsenic guidelines development), since these results suggest that low DO can increase arsenic toxicity and, if not properly considered, lead to an underestimation of arsenic toxicity in aquatic ecosystems.

# The definitive identification of lead shotshell as a major source of lead exposure in native communities

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In the mid 1990s, a breast-feeding study by members of the team revealed an association between lead concentrations in maternal blood and the consumption of traditional foods obtained by hunting. A similar relationship was observed between cord blood lead levels of newborns and the maternal intake of wild meat, especially waterfowl. Subsequently, radiographic evidence was obtained that demonstrated the presence of lead pellets in the gastrointestinal tract of adult First Nation Cree living in the James Bay region of Ontario, as well as for lead fragments in waterfowl tissue bagged with leaded ammunition. With this as a background, our research project had two objectives. First, to compare the body burdens of lead (as measured by whole blood lead) of 50 adult females and 50 adult males living in each of two James Bay First Nation Cree communities, namely Fort Albany and Kashechewan. A comparison group of 50 adults (equal number of males and females) was established in Hamilton, Ontario. The second objective was to compare stable lead isotope ratios in blood samples with those in lichens and in leaded ammunition. The purpose of this comparison was to pinpoint the primary source of lead that the individuals are exposed to. This is possible because different sources of the metal lead, such as that used in manufacturing leaded ammunition and that present in the general environment, have a different signature of lead isotopes. (Lead stable isotopes are lead atoms of slightly different mass; they differ in the number of atomic particles contained in their nuclei.) In this comparison, lead in lichens reflects the type of lead present in the general environment and thus that delivered by the aerial route from industrial sources usually by the aerial route.

The results obtained clearly illustrate that blood concentrations of lead are considerably higher in the native communities compared to Hamilton residents of comparable age, by a factor of one-and-a-half (females) or two (males) based on geometric means. In Fort Albany and Kashechewan, more females (10% *versus* 4% in Hamilton) have lead concentration above the level of medical concern specified for females of reproductive age as one hundred micrograms per liter. (The same level of medical concern applies to children.) In a subsample of hunters, the lead concentrations increased substantially (by as much 40%) when comparing values before and after the hunting season. In fact in some individuals, the after-the-hunt levels reached those observed occupationally. An inspection of the lead isotope ratio data supports the interpretation that leaded ammunition is a primary source of lead. Our findings have been shared with members of the native communities in support of a concerted effort to limit the exposure to lead from the use of leaded ammunition and from the consumption of meats contaminated with lead shot or fragments.

# Transport of metals from mine tailings impoundments and release to surface waters

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National Water Research Institute, Environment Canada
Contributing Partner: D.W. Blowes
Department of Earth Sciences, University of Waterloo

Mining activities result in the production of tailings which are disposed of in large piles or impoundments. Tailings typically contain high concentrations of sulfide minerals that are not economical to extract, such as pyrite and pyrrhotite, and trace concentrations of other metal sulfides, such as sphalerite and galena. Refinery wastes, the byproducts from processing ores to produce metal commodities, often are disposed with the tailings. Over time, the refinery wastes break down and the sulfides in the waste oxidize, releasing acid, dissolved metals including Fe, Zn, Cu, Ni, Co, sulfate and other elements, such as arsenic, to infiltrating precipitation water. As this low-quality water migrates through the tailings piles or impoundments, a series of reactions occurs, leading to the attenuation of some metals and neutralization of acid. Eventually, at many mine waste sites, very high concentrations of metals and other substances migrate from the impoundment, leading to degradation of groundwater and surface water quality. In the absence of remediation, unacceptable concentrations of acid and dissolved metals will discharge from the sites long into the future. This project involved a combination of field studies, laboratory studies and model calculations to improve predictions of long-term loadings of metals and other substances from mine tailings impoundments. Hydrogeological and geochemical field investigations were carried out at tailings impoundments at three base-metal mines (Timmins, Ontario, Sudbury, Ontario and Flin Flon, Manitoba) and a gold mine (Red Lake, Ontario). Complementary laboratory studies were conducted to provide information on the metal uptake processes and to evaluate the long-term potential for the metals to be attenuated at a given site. At all sites investigated, very high concentrations of sulfate, trace metals or other dissolved constituents, such as arsenic, were observed in the tailings pore waters. At the older sites investigated, severe degradation of groundwater and surface water quality was observed. Model calculations, using a fully coupled reactive solute transport model calibrated using the field and laboratory data, were used to predict metal loadings to adjacent water bodies. The model predicts that concentrations of dissolved substances in the discharging waters will increase in some cases for many decades and then will be followed by a gradual decline which will last for many years after. The results can be used to provide a more accurate projection of costs required to remediate existing sites, and to develop improved tailings disposal practices.

# Bioavailability of Arsenic in Yellowknife

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This project assessed the ecological and human health risks of Arsenic. The research explored recent evidence that certain types of arsenic exposure may be more harmful than previously believed. The research was conducted in Yellowknife, NWT, which like many areas in Canada, has elevated total arsenic concentrations as a consequence of both natural (arsenic-bearing minerals) sources and historical mine and smelting inputs. This research provided information may be used in risk assessments, and developed procedures that can be used for other arsenic contaminated sites in Canada and the world.

# Cycling of mercury in Kejimkujik National Park

Andrew Rencz

Kejimkujik Park, Nova Scotia is noted for having the highest mercury concentrations in loon blood in North America. The work has been targeted to address unanswered questions on sources and processes thought to account for the anomalous levels of mercury. Sources and transformation of mercury in natural ecosystems are poorly understood and a multi-disciplinary approach is needed to understand the complex cycling of mercury in aquatic and terrestrial environments. The multidisciplinary group comprises biologists, geologists, chemists, limnologists, remote sensing and GIS experts.

Fieldwork was designed to collect samples, for chemical analyses, from a variety of media in a range of environments in and adjacent to the Park. The various media sampled include: vegetation, soils, rocks, lake sediments, stream water, lake waters and ground water. Hg flux measurements were also taken to characterize the exchange between vegetation, soils and water with the atmosphere. The objectives were to characterize the pools of Hg, quantify the flux between the various pools and describe the various geochemical processes underlying the movement of Hg.

The project, over the first two years, has characterized the distribution of Hg in the various pools (media) and the spatial variation of Hg across the Park. There is variation across the Park in levels of Hg; however concentrations of Hg are not significantly higher in most media than in other areas across Canada. The areas of higher total Hg concentrations are related to the underlying geology. The incorporation of the multidisciplinary data into a mass balance budget is still in progress but initial calculations indicate: significance of atmospheric input, and several unexpected results: including: the importance of groundwater as a source of MeHg to lakes, and the importance of vegetation and volatilization as a pathway for mercury. Study indicates the need to produce a mass balance for MeHg.

Although, as noted, the levels of Hg are not significantly higher in most media, the levels of mercury are significantly higher in upper parts of the food chain, notably the loons. This points to the importance of biomagnification of Hg up the food chain and of the importance of the methylation process. To address the significance of MeHg and its relationship to organic matter several new areas of research have been incorporated into the project: these include the analysis of microbiological processes in mercury cycling and transformations, and sedimentation processes as a sink for mercury. Research in this project has shown the importance of wetlands to the production and transport of methyl mercury, the contribution of soils as a mercury source, and the significance of mercury flux from various media.

The results from this project are important since they will help to clarify the relative importance of atmospheric deposition, geological sources, and geochemical, biological and photochemical processes on mercury concentrations in aquatic and terrestrial systems. The data collected will allow for an enhanced knowledge base for site-specific risk assessments concerning mercury and to provide an improved framework for determining effective risk management strategies. The research also indicates the possibility of using remote sensing to identify potential areas of toxic element (mercury) risk. The mass balance work will help to clarify the relative significance of inputs and outputs within the Kejimkujik ecosystem.

# Wildlife toxicology of lead

Scheuhammer, A., Campbell, D., Martin, P., Thomas, V., and Wickstrom, M.

Our research seeks to assess the impacts of environmental lead contamination on Canadian wildlife; to study the issue of elevated lead exposure and poisoning in upland game birds and their predators; to study some potential environmental effects of lead deposition at outdoor shooting ranges; and to use stable lead isotope analysis of animal tissues and environmental media to distinguish different sources of lead exposure in Canadian wildlife. By examining hawks and owls found dead in various locations across Canada, we have been able to determine that a small but significant proportion of these birds have elevated lead exposure, and some have even died of lead poisoning. Similarly, we have observed elevated lead exposure in 11% of young-of-the-year Hungarian Partridge and 5% of young-of-the-year Sharp Tailed Grouse, two species of upland game bird from Prairie Canada. In waterfowl, such incidences of high lead exposure would not be surprising, because waterfowl are well known to ingest spent lead shotgun pellets from the bottoms of marshes and lakes. However, lead shot ingestion in upland game birds has not been well studied previously, and has been assumed by many biologists and wildlife managers to be infrequent. Thus, it is significant that our research shows that Hungarian Partridge bones with high lead accumulation reveal a pattern of lead isotope ratios consistent with lead shot ingestion. Lead isotope analysis further revealed no evidence that these upland game birds were exposed to high levels of environmental lead from other suspected sources, such as lead from past gasoline combustion. Our evidence thus far indicates that the most probable source of high lead exposure in these upland game bird species and their predators (hawks and owls) appears to be the ingestion of Pb shot, rather than exposure to other potential sources of environmental Pb. The use of stable lead isotope analysis, such as we are employing in this study, is particularly important and useful for the development of sound environmental and wildlife management policies, for which it is essential to understand the relative importance of different potential sources of environmental lead exposure.

# Estimation of mercury emissions from natural sources processes in Canada

Schroeder Bill, Edwards Grant, Rasmussen Pat, Beauchamp Steve, Poissant Laurier

Mercury is ubiquitous in the environment, being derived from a wide variety of natural as well as human activity related sources (i.e., anthropogenic). Over the last 25 years, much effort has been expended to frequently update and continuously improve anthropogenic mercury emission inventories in Canada and other industrialized countries. So far no concerted analogous effort has been made, or even formally proposed, anywhere to address the much debated question concerning the relative magnitude/importance of natural versus anthropogenic Hg emissions. Thus large uncertainties remain concerning the relative magnitude of the contributions from the numerous natural and anthropogenic sources/process releasing mercury into the environment.

The mid-1990's marked a turning point in Canada's approach to dealing with the lack of information and the degree of uncertainty associated with the (relative) significance of natural sources as contributors to the atmospheric mercury burden both in Canada as well as globally. This research project is a collaborative effort including agencies within environment Canada, including the Meteorological Service of Canada, as well as the Quebec and Atlantic regions. Outside agencies involved include University of Guelph, Geological Survey of Canada, and Health Canada.

The overall objectives are to assess the role of the atmosphere in Hg cycling in natural ecosystems, to provide input to atmospheric Hg models, and to provide information which will help to differentiate natural vs. anthropogenic sources of Hg in the Canadian environment and assess their relative importance. TSRI#105 has undertaken to address the following critical information gaps associated with natural emissions/processes in the environment; 1) the lack of representative and reliable data on natural emissions/sources/processes emitting volatile mercury species to the atmosphere in Canada (and elsewhere, recognizing that transboundary transport of Hg can and indeed does occur) and 2) evaluation (or development) of models/procedures applicable to scaling up experimental results, obtained from in-situ (field) flux measurements, to regional and national scales.

The project objectives have been successfully met. Due to the complexity of the biogeochemical cycling of Hg from natural sources in the environment, a multi-disciplinary approach, with three teams of scientists was used to address information gaps related to the lack of representative and reliable data on natural sources and the processes resulting in emission of volatile Hg. Team A undertook extensive laboratory and field studies. Laboratory studies were aimed at improving both dynamic flux chamber and micrometeorological techniques to determine Hg fluxes from natural sources. The field studies were designed to continue data collection in contrasting geological settings and climatic regimes across Canada. Team B conducted *in situ* Hg surface/air exchange measurements over various landscapes in the Quebec region and team C conducted research in the Atlantic region in contrasting natural landscapes consisting of marine, freshwater and terrestrial ecosystems. Within these types, underlying geology, surface coverage by remnant glacial tills, vegetation cover, climate, moisture and organic content are confounding factors influencing the study of natural Hg emissions.

Resulting from this intensive effort over the past three years are extensive sets of data that have provided new insights into the sources/processes controlling the movement of gaseous mercury in our environment. Through modeling and associated scaling up activities a better understanding of the contribution of natural sources to the global mercury budget has been gained.

# Mercury in forest watersheds after fire or logging removal

William M. J. Strachan, Ellie E. Prepas, Weiping C. Chen, Ian D. Campbell, Stephan Gabos

In forests, the vegetation represents a surface area that is much larger than the land itself and absorbs the mercury from the atmosphere, from the air and from the rain. Leaves fall to the forest floor and remain as litter eventually becoming soil. The mercury that was part of the litter is partially retained there and partially leaches out with rain, enters the lakes and streams, and accumulates in the fish and other biota there. A watershed drainage area typically comprises the lake (5-35%) and the forest itself; the mercury accumulates in the lake. When the forest cover is removed through fire or intensive harvesting of timber, the runoff characteristics change and the objective of the study was to see how the concentrations of mercury in the streams and fish would change with these removals.

A number of lakes with a known history of burning and a separate stream were investigated in the Swan Hills area of northern Alberta; one of the lake and the stream watersheds were subsequently harvested and; by chance, the harvested lake was extensively burned. All of the analytical results are not complete and the comments here are tentative and are not the limit of what may be concluded from data yet-to-come.

Mean surface water concentrations in 1999 were fairly uniform throughout the area — 1.3-1.5 ng/L — although the number of lakes was limited. In 2000, a number of previously (2 years) burned lakes were compared with reference lakes where there had been no disturbances from fire or harvesting. Mercury concentrations in the burned lakes were higher (1.4 ± 0.2 ng/L) than in undisturbed lakes (0.8 ± 0.2 ng/L). The pattern of mercury in invertebrates in these lakes was ambiguous - most taxa did not show differences although at least one (dragonfly larvae) showed higher levels in burned lakes (57 ± 4 ng/g vrs 39 ± 5 ng/g). A contaminated lake (i.e., one with a fish advisory on consumption) sampled at the same time had even higher levels in this taxa (84 ± 10 ng/g). It has not been possible to collect significant numbers of top predator fish in these lakes but efforts are being made to do this for the harvest/fire lake and one or more reference lakes.

Results for before and after comparisons of the water quality in the two systems which were harvested and the one that was burned are not available at this writing. The burned system has also been the subject of a more detailed recovery study, first samples being collected some few weeks after the fire (May 2001) and continuing on a biweekly basis until freeze-up (October 2001).

It is anticipated that the study will yield observations with significance for the issuing and removal of fish advisories. The extent of the increase in the mercury water concentrations with resulting increases in levels in the invertebrate food sources of fishes and the fish themselves will assist in predicting the likelihood of fish levels exceeding food guidelines. The prediction of the recovery times of lake systems from these elevated concentrations will be useful in designing fish sampling programs to determine whether the advisories need to continue.

# Validation of the diffusion gradient in thin-film (DGT) technique for assessment of cadmium and lead bioavailability in the aquatic environment

Michael Twiss, André Tessier, Lise Rancourt and Richard Goulet

Toxicity of trace metals to aquatic organisms depends on the specific forms of the metals in natural waters. One technique for measuring metal forms in the natural environment, called DGT (Diffusion Gradient in Thin-film), has been proposed recently.

Our main objective is to verify if the DGT technique can be used to determine metal forms and to predict the accumulation of metals by aquatic organisms in natural waters typically encountered in Canadian waters. Figure 1 depicts a cross-section of a DGT probe. It comprises a metal binding resin that binds metals rapidly and efficiently and a diffusion gel that controls the transport of metal forms from the external solution to the resin gel. The quantity of metal accumulated ($Q$; mol) into the resin gel is related to the concentration of metal forms in the solution ($C_b$; mol $l^{-1}$) by the following equation:

$$Q = \frac{{}^{g}D \cdot A \cdot t}{\Delta g} \cdot C_b$$

where ${}^{g}D$ ($cm^2 \cdot sec^{-1}$) is the diffusion coefficient of the metal in the diffusion gel, $A$ is the surface ($cm^2$) of the diffusion gel exposed to the external solution, $t$ is deployment time (sec) and $C$, ($\mu g \cdot l^{-1}$) is the concentration of metal species in the external water, and $\Delta g$ is the thickness of the diffusion gel (cm). A DGT probe looks a lot like a hockey puck.

Validation of the DGT technique for natural waters involves: i) comparison of the results obtained by this technique with those obtained by other techniques *in situ* and in the laboratory; ii) determination of potential interactions of metal forms with the diffusion gel that could compromise the use of the DGT technique in natural waters. Results to date indicate that some metal forms interact with diffusion gels - this behaviour is non-ideal and it challenges an unambiguous interpretation of results from DGT probes in natural systems. Nevertheless, our research continues to better understand the chemical forms of metal that the DGT is measuring since the technique has much promise.

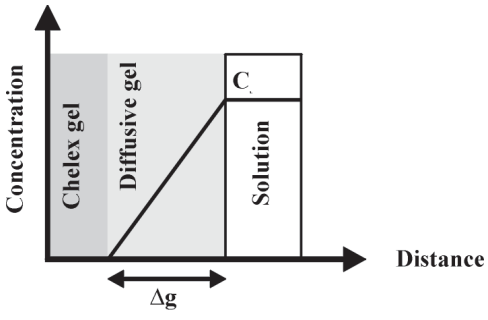


Figure 1. *Schematic representation of a solute concentration gradient in a DGT device. Δg is the thickness (cm) of the diffusion gel.*

# Assessment of toxicological risks of environmental contamination by manganese

Zayed Joseph[1], Carrier Gaétan[1], Kennedy Greg[2], Butterworth Roger F.[3], Hazell Alan[3], Mergler Donna[4]

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The central nervous system represents an important target for manganese (Mn) intoxication which may cause neurological symptoms similar to Parkinson's Disease in humans. With the addition of methylcyclopentadienyl manganese tricarbonyl (MMT) in unleaded gasoline which leads to fine particle Mn emissions, Mn has once again attracted attention. Micro-environments with high traffic density show higher Mn concentrations. It has been suggested that combustion of MMT may be a significant source of exposure to inorganic Mn in urban areas, since it is emitted from the tailpipe. One of the crucial questions is whether a low increase of Mn contamination resulting from the widespread use of MMT could lead to neurotoxic effects. This research measured inhalation pharmacokinetics and neurobehavioral toxicity of different Mn compounds in an animal model.

A total of 270 Sprague-Dawley rats were exposed by inhalation to different levels of the different Mn compounds. The exposure took place in inhalation chambers for a period of 13 consecutive weeks, 5 days per week and 6 hours a day. Results show that brain tissue concentrations are dose-dependent. Brain is a target site for Mn accumulation. Moreover, some neurotoxicological effects (neurobehavioral status, histopathological damage and associated muscular tremor) were observed, mainly at high exposure levels. These results should contribute to the successful implementation of evidence-based risk assessment approaches related to the use of MMT in the Canadian gasoline.

# Endocrine Disrupting Chemicals (EDCs)

Endocrine disrupting chemicals are substances which have the ability to alter or disrupt hormone or endocrine sys-tems. Since its launch in 1998, the TSRI has allocated over $6.1 million in funding 17 research projects in the area of EDCs.

In fiscal years 1999-2000 and 2000-2001, applicants were asked to accelerate research activities designed to establish an adequate understanding of the implications of EDCs.

### The priority knowledge needs in fiscal year 1999-2000 were:

- To identify outcome measures that are predictive of adverse effects in ecosystems and humans exposed to various EDCs;
- To investigate the linkages between ecological and human health effects resulting from exposure to environmental levels and mixtures of EDCs;
- To develop screening tools to evaluate the potential physiological effects of EDCs in the environment;
- To determine the sources of and sinks for EDCs in the environment and prioritizing substances for further testing;
- To characterize dose-response relationships for environmental and human health effects in order to determine risks associated with EDCs;
- To determine mechanisms of action for EDCs and environmentally relevant mixtures where regulatory decisions are required; and
- To determine the pharmacokinetic and pharmacodynamic effects of EDCs where regulatory decisions are required.

### The priority knowledge needs in fiscal year 2000-2001 were:

- To identify adverse ecological or human health effects resulting from exposure to environmental levels and mixtures of endocrine disrupting chemicals;
- To determine the identity, sources and fate of endocrine disrupting chemicals in the environment; and
- To develop standardized methods that are predictive of endocrine disrupting activity in the environment for use in the screening of chemicals for endocrine disruptive properties and for prioritizing chemicals for further, more intensive investigation.

### In fiscal year 2001-2002:

There were no call for proposals in fiscal year 2001-2002, therefore, no knowledge needs were developed for any of the priority research areas. However, there were still ongoing research projects which were previously approved in the 1999-2000 fiscal year.

# Effects of orchard pesticides on terrestrial wildlife

Christine Bishop, Pamela Martin, John Elliott, Tony Williams

The destruction of habitat and the continued decline of uncultivated lands make semi-natural areas such as apple orchards attractive foraging and nesting sites for wild birds. However, apple orchards are the crop most intensively sprayed with pesticides in Canada and receive as many as 11 applications per season with a variety of compounds. Spraying occurs from early April to mid-August, coinciding with the breeding season of wild birds. In addition, past spraying with DDT insecticide has contaminated of the entire food chain, resulting in very high concentrations in songbird eggs.

Effects on reproduction, endocrine and immune function in wild birds living in orchards have been reported in several studies. In our study we assessed effects of DDT and currently used pesticides on reproduction, endocrine and immune function in wild birds inhabiting orchards and tried to compare these effects to those observed in a tame bird species that were exposed to DDT and known doses of orchard pesticides, as well as those seen in laboratory studies on captive wild birds from DDT-contaminated and uncontaminated sites.

We erected nest boxes for tree swallows in several sprayed orchards and unsprayed pasture sites in southern Ontario and observed reproductive success. In each nest, we measured DDT in one egg; thyroid stress hormones in 2 chicks; and immune function in one chick. There were no differences in number of chicks produced per nest between orchard and pasture sites. Eggs in orchard sites had much higher DDT levels, and there were differences in thyroid hormone levels between orchard and pasture sites, that related to levels of DDT in the eggs. Thyroids of orchard chicks had developed abnormally. There were no differences in stress hormones or immune responses. Because thyroids are important for the growth and development of birds, these changes could be important to the birds nesting in orchards.

American robins taken from nests in highly contaminated orchards in the Okanagan valley also had slightly altered thyroid hormone activity and had different behavior during the breeding season than did robins from the cleaner lower mainland of British Columbia, when raised in captivity. Orchard robin males sang less and orchard females laid and hatched fewer eggs than did lower mainland birds.

Captive zebra finches dosed with DDT and 2 currently used orchard pesticides did not show any differences in the number of chicks produced or growth of chicks from undosed birds. There were some differences in blood cells, suggesting some interference with the immune system of the birds as a result of DDT and pesticide exposure.

The combination of contamination with old pesticides such as DDT and exposure to current pesticide spraying may result in alteration of the endocrine and immune systems of birds using orchards for breeding habitat.

# The effects of endocrine disrupters on sea water adaptability, growth and survival of salmon smolts

Scott Brown, Wayne Fairchild, Kats Haya, Geoff Eales, Deborah MacLatchy, Jim Sherry, Don Bennie, Kent Burnison, Robert Evans

**Background.** A recently published paper showed that chemical use between 1975 to 1985 may have affected wild salmon populations in Atlantic Canada. The concern was that during this time period, one insecticide formulation, Matacil®1.8D, sprayed to control damage from the spruce budworm contained high concentrations of a compound called 4-nonylphenol. Nonylphenols are known to exert estrogenic effects in fish and are classified as hormonal mimics or "Endocrine Disrupting Substances". It was recognized that spruce budworm spraying also coincided with the final stages of salmon smolt development (the process whereby young stream-dwelling salmon become physiologically adapted for survival in seawater). When looking at exposure of this life stage, adverse effects on subsequent salmon returns were apparent with low fish catch coinciding with areas where Matacil®1.8D was applied. To identify an outcome that may be predictive of adverse effects for migrating salmon populations, we examined whether parr-smolt transformation in Atlantic salmon was sensitive to the low levels of nonylphenol and estrogens that may occur from current discharges into rivers supporting sea-run salmon stocks. To help estimate the risk from these discharges, the project examined important periods for exposure and doses for these substances. Most current sources of environmental steroids are from sewage effluents, agricultural wastes or industrial effluents, and the ability of these mixtures to produce effects similar to those observed for pure compounds was evaluated.

**Growth and Survival.** Our laboratory experiments showed that growth, based on weight gain in treated salmon from transfer to salt water (mid-June) to October, was bimodal. This suggests that some of the population can be affected by brief (2-5 day) exposures to estrogen or nonylphenol in water. There were no treatment related effects on survival during long term grow-out in seawater. Wild smolts, collected upstream in the NW Miramichi River, were caged at various locations in the Miramichi and in Tabusintac (reference) estuaries. Bimodal growth patterns were evident in fish regardless of caging site. However, relative to the fish caged at the Tabusintac reference site, the proportion of small fish was significantly greater for the fish caged at three locations near effluent sources in the Miramichi estuary for the 2000 smolt run.

**Biochemical, Histological and Endocrine Parameters.** Exposed and reference fish were assessed for osmoregulatory parameters, growth factors, levels of steroid hormones and the egg yolk protein, vitellogenin, a commonly assessed biomarker for exposure to estrogenic substances. Nonylphenol and estrogen treatment produced few effects on osmoregulatory measures but altered indicators of exposure (vitellogenin) and parameters that may be predictive of the observed growth deficits (thyroid hormones and insulin-like growth factor).

**Exposure measures.** Polycyclic aromatic hydrocarbons (PAH), numerous trace metals and nonylphenol content of water and sediment from the Miramichi were analyzed. PAH levels in river water were low but pulp mill effluent and sediments at Chatham contained some PAHs. Trace metal analysis showed ion and nutrient enrichment in pulp mill and sewage effluents. Water and sediment samples collected from the Miramichi estuary generally showed low concentrations of nonylphenol. However, there was low but detectable estrogen levels associated with sewage and other inputs.

**Ecological Significance of Impaired Growth.** Marine survival of given smolt year classes have been related to early ocean growth. Atlantic salmon smolts exposed to nonylphenol and estrogens in our laboratory and field experiments have a greater proportion of slow growing smolts in the months after introduction into seawater. If this also occurs in the wild following exposures to active agents in current discharges, then lower adult returns may be expected.

# EDCs in municipal sewage effluents

B. Kent Burnison, Mark Servos, Jim Sherry, Bill Lee, Glen Van Der Kraak, Chris Metcalfe

**Background.** Sewage treatment plants (STPs) are designed to remove organic and inorganic contaminants from municipal sewer wastewater. However, STPs do not function at 100% efficiency. Some of the organic contaminants that have been shown to be present in final STP effluents are chemicals that can cause harmful effects to the endocrine systems of aquatic organisms. Collectively, these chemicals are referred to as endocrine disrupting compounds (EDCs). The endocrine system consists of glands and the hormones they produce that guide the development, growth, reproduction, and behavior of human beings and animals. Some of the responses that have been observed in fish below STPs are the induction of vitellogenin (egg yolk protein) in the male blood, the development of eggs in the testes and even females exhibiting secondary male sexual characteristics. A number of significant information gaps have been identified in the assessment related to endocrine disruption which are preventing a risk characterization being completed and will affect the ability to prepare proper risk management strategies. A commitment to conduct research on endocrine disruptors was recently made by the Canadian Federal Government under the amended Canadian Environmental Protection Act (CEPA). This proposal is designed to address the critical information needs identified in these current government programs and provide knowledge required to make sound science based decision on future regulatory needs, directions and options.

**Current Status.** Our field studies have shown that some STP effluents in southwestern Ontario induce vitellogenin in caged male rainbow trout. In addition, eggs have been observed in male gonad tissue in free-living fish below sewage outfalls. The chemicals responsible for these observations in fish have been isolated and identified. Using a combination of chemical and biological techniques, we have shown the presence of the natural female hormones (estradiol and estrone), the synthetic estrogen (ethynylestradiol in birth control pills), the male hormone testosterone, and industrial chemicals such as bisphenol-A (produced in the making of polycarbonate plastic, epoxy resins and other products) and alkylphenolics (found in detergents, shampoos, cosmetics, etc.). All these compounds are well known EDCs. One of the alkylphenolics is called nonylphenol polyethoxylate and it biodegrades as it passes through the sewage treatment plant. The breakdown products are more estrogenic than the starting material. The concentration of these breakdown products is relatively high in STP effluent, therefore proving the estrogenic potency of these compounds is very important in the development of risk management policies. Our laboratory experiments have shown that one of the breakdown products, nonylphenol carboxylate, does not bind to the human estrogen receptor; shows weak binding to the trout estrogen receptor; and does not effect the sexual characteristics of a test fish. Another breakdown product, nonylphenol ethoxylate was shown to be estrogenic in our tests, but much less than another breakdown product, nonylphenol. A 21-day experiment testing nonylphenol, nonylphenol carboxylate and nonylphenol ethoxylate at five concentration levels has been completed and awaits chemical and biological determinations. These results are directly applicable to PSL-2 Risk Assessment of Nonylphenol/Nonylphenol ethoxylates.

# Adverse reproductive effects of exposure to dioxin-like endocrine disruptors

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This project was designed to study the reproductive effects of polycyclic aromatic hydrocarbons (PAH). These chemicals are ubiquitous environmental pollutants and toxicants produced as byproducts of combustion of fossil fuels. A major source of human exposure is from automobile exhaust, furnace gases and especially from cigarette smoking, both active and passive. PAH have been shown to have adverse health effects by binding to the aryl hydrocarbon receptor (AhR) resulting in the expression of genes that control enzymes that can metabolize PAH to chemicals that bind to DNA and cause mutations or cell death. The results of such metabolism include heart disease, cancer, and reproductive dysfunction including damage to human eggs and sperm. We used 2 mouse models to investigate the mechanism of adverse effects on sperm production and the toxic effects of PAH on human eggs. In addition, we determined if a recently discovered natural AhR antagonist, resveratrol, produced in response to fungal pressure by grapes and other spermatophyte plants and found in some red wines, could prevent any observed adverse effects of PAH.

We have determined that increasing doses of the cigarette smoke component, benzo(a)pyrene (BaP) reduced sperm concentration by a combination of necrosis and programmed cell death. Programmed cell death requires genetic programming for death usually as a result of DNA damage. We were able to demonstrate that BaP increased AhR-related enzyme induction and DNA damage. We also determined that the death of sperm cells as a result of PAH administration could be blocked by co-administration of resveratrol, leading to a potential therapeutic measure to prevent male infertility.

We have also performed preliminary experiments that involved transplantation of human ovarian cortex into NON-SKID (immunocompromised) mice. In this experiment, the grafts successfully implanted and the human ovarian tissue containing healthy primordial, primary and secondary follicles was observed. The effect on ovarian follicles of treating the mice with 7,12-dibenzo(a)anthracene (DMBA) diol epoxide, another cigarette smoke component was determined. The expression of cell death genes was determined in the follicles and in the human eggs contained in the follicles. We demonstrated that DMBA induced egg cell death in the human ovarian cortex transplants through increased expression of cell death genes such as *Bax*. This finding could explain the observation that women who smoke undergo menopause prematurely. We also demonstrated in preliminary studies that resveratrol could block this induction of cell death genes.

Finally, we have examined the effect of administration of PAH to pregnant or lactating mice to determine whether these chemicals have an effect on the offspring and to evaluate the relative toxicity of transplacental and lactational transfer of PAH on future reproductive function. So far, we have shown that the chemicals cross the placenta and are also delivered to the mouse pups in breast mild. In both cases, there was enzyme induction in the mouse pups similar to that described in the above experiments.

The experiments funded by this TSRI grant may have important implications for both male and female infertility. In addition, resveratrol, a natural substance found in some red wines, may offer protection against the adverse reproductive effects of smoking and other airborne environmental aromatic hydrocarbons.

# To Document Neonatal Exposure of Endocrine Disrupting Chemicals in Human Amniotic Fluid

Siu Chan, John Jarrell
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Many chemicals are known to interact with the hormonal system, especially in wildlife, but there are circumstancial evidence that humans are also affected. These chemicals are generally known as endocrine disrupting chemicals. Examples are polychorinated biphenyls (PCBs), pesticides and phytoestrogens, which are naturally occurring plant chemicals. The goal of this Project is to investigate whether neonates are exposed to these chemicals. We have developed very sophisticated analytical procedures that will allow us to measure the contaminants down to very low levels (sub-part per billion) in body fluids using small amounts of sample, usually one to two ml.

Our study subjects were women from Southern Alberta, 35 years of age or over. They undertook amniocentesis for genetic investigations in the second trimester At the time of the procedure, a blood sample was also collected from the subject. We performed chemical analyses on these samples.

Our preliminary results showed that no PCB compounds or pesticides were detected in amniotic fluids. However, there were phytoestrogens in the majority of the samples. The phytoestrogens we measured were daidzein and genistein, which are derived primarily from soy products. Their concentrations were much higher than that of the female sex hormone at the same stage of pregnancy. However, the female sex hormone is much more potent, and whether the presence of these chemicals has any health effects on the fetus remains to be seen.

Many mother's blood samples were also positive for the phytoestrogens, usually ten times the concentrations of those in the amniotic fluids. Many samples also had DDE, a metabolite of the pesticide DDT, the fungicide hexachlorobenzene and many PCB compounds. It is also of interest to investigate whether the presence of these compounds in the mother's blood has any health consequence to the baby.

We also collected cord blood at birth and a breast milk sample from the mother at a later date. It is expected that many of these compounds are also present in the mother's milk and they will be passed on to the baby through breastfeeding. Indeed in another study we have found PCB compounds, DDE and hexachlorobenzene in many breast milk samples obtained from Albertans. On the other hand, we did not find many milk samples with phytoestrogens.

An environmental questionnaire was requested from each subject during genetic counselling prior to amniocentesis. They were asked about their dietary intake, specific diets, beverage consumption, medical condition and chemical exposure in the previous year. Statistical analysis was performed on entries in this questionnaire and test results. One interesting preliminary finding was that there was a correlation between inferility in the previous year and the presence of phytoestrogens in the amniotic fluid. This Project is still ongoing and will eventually enlist three hundred subjects.

# Time to pregnancy and environmental contaminants

Donald Cole

**Purposes** This study sought associations between parental levels of both persistent organic pollutants (POPs) and toxic metals, biological indicators of reproductive health such as reproductive hormone levels, and the time taken for couples to get pregnant, the time to pregnancy (TTP). TTP can be affected by substances which influence fertility in fathers and mothers.

**What we did** We worked with questionnaire data and biological samples from 62 couples having their first child who were planning to deliver at St. Joseph's Hospital in Hamilton, Ontario. We measured concentrations of organochlorine pesticides, polychlorinated biphenyls (PCBs), and metals in the blood of mothers and fathers, as well in the umbilical cord blood and placentas of their newborns. In pooled samples of blood we measured dioxins, furans and certain kinds of PCBs. We also measured reproductive hormones in the mothers' blood and the activity of certain enzymes in the placentas which are known to increase in abundance and activity when exposed to dioxin and other endocrine disruptors. In statistical analyses looked at the relationships among 1/ contaminants within a person and 2/ contaminants in mothers and fathers and mothers and their babies. We also compared the levels of the contaminants, the reproductive hormones and the receptor activity by TTP groups (i.e. one month, two to six months and six or more months).

**Results** A number of POPs (aldrin, chlordanes, nonachlors, endosulphans) were detected in less than 50% of mothers, babies and fathers. Others (lead, mercury, PCBs, DDE & DDT, mirex, hexachlorobenzene, pooled dioxins and furans) were detected more often. The relative amounts of contaminants within a person were strongly correlated. Fathers in general had greater contaminant loads than mothers and the relative amounts of each contaminant were significantly different, which suggests that men and women deal with contaminants in a fundamentally different biological fashion. Contaminant levels in umbilical cord blood were very low. Contaminant levels across TTP groups were similar as were mother's hormone levels. Measures of placental receptor activity were low and similar across TTP groups.

**Importance** Our research indicates that measuring a smaller number of indicator substances closer to the time of conception may be an adequate measure of contaminant exposure in future studies and that maternal and paternal contaminant dynamics are significantly different. The lack of clear associations between low levels of POPs in biological tissues and delays in TTP should somewhat re-assure the general public. However, in order to participate in our study a couple had to have become pregnant, thus ruling out the completely infertile population. We also had a relatively small sample size and the participants in our study have very low levels of contaminants so our work does not preclude such contaminant effects that might be detected in larger, more highly exposed populations.

# Human daily intake and mammalian immunotoxicity and reproductive toxicity of organotin

Cooke Gerard, Robaire Bernard, Hales Barbara, Trasler Jacquetta, Morales Carlos, Hermo Louis, Brawer James, Cyr Daniel, Tryphonas Helen, Forsyth Don

Tributyltin (TBT) is used as an antifouling agent in marine paints and causes imposex in marine gastropods, where the females develop male sexual phenotypes resulting in infertility. Human exposure to TBT occurs, primarily, through the consumption of contaminated food and thus, the entire Canadian population is at risk. The tolerable daily intake (TDI) for TBT is 0.25 µg/kg body weight/day, based on its toxicity to the immune system. The actual intake of TBT by Canadians through food is unknown. Thus, we pursued three lines of investigation to determine; a) the amounts of organotins that contaminate the Canadian food supply; b) the effects of continual exposure to TBT from early pregnancy to adulthood on the immune system of rats and; c) the effects of TBT exposure during pregnancy on the development of the rat reproductive tract.

We developed a method to detect organotins in commercial human foods. Several fresh and preserved seafoods were found to contain TBT at levels up to 300 ng/g. Di- and monobutyltins were present in fewer samples and at lower levels compared with TBT.

When pregnant rats were treated orally with TBT at 0, 0.025, 0.25 and 2.5 mg/kg body weight/day from day 8 of gestation, through lactation and then the pups dosed until adulthood with the same dose as their mothers, all TBT doses caused a reduction in body weight of the female pups. Ongoing evaluation of the pups' immune system has revealed that the proportion of blood cells responsible for eliminating neoplastic cells and viral infections (natural killer (NK) cells) was significantly increased. NK cells had increased activity, especially in male pups, at all TBT doses. The significance of these effects on NK cells has to be evaluated in conjunction with other immunological endpoints currently being studied.

Oral administration of TBT at 0, 0.25, 2.5, 10 or 20 mg/kg/day during pregnancy only, followed by sacrifice on day 20 of gestation (just before birth), revealed that the dams had significantly lower serum thyroid hormone levels. The fetuses also had skeletal malformations manifested as an increase in unfused bones. Fetal ovaries and testes had abnormal appearances under the microscope; the numbers of germ cells were decreased. TBT altered the expression of genes in the fetal gonads in a gender-dependent manner: in fetal ovaries, TBT down-regulated many genes and up-regulated only a few, but in the fetal testes, many genes were up-regulated by TBT and only a few were down-regulated.

When the same study was done and the pups followed to adulthood, TBT caused a delay in male sex development (separation of the prepuce), decreases in testis and prostate weights and abnormal sperm motility. In female pups, markers of sexual development were also delayed (vaginal opening, first estrus and first diestrus). The gene expression profile of adult gonads was also altered and a gender difference was evident, with more genes being affected by TBT in the testis than in the ovary. As a result of altered gene expression, there were long-term adverse effects on endocrine function caused by exposure to TBT in utero.

Thus, there is significant contamination of human foodstuffs with organotins; the mammalian immune and reproductive systems are susceptible to low doses of TBT and TBT causes gender-dependent alterations in gene expression. In fact, there was no dose of TBT that was without adverse effects. Our data indicate that the safety factors for determining the human tolerable daily intake of TBT may have to be revised.

# Reproductive toxicity of trichloroethylene

P. G. Forkert, R. Tardif, M. Wade, N. Tanphaichitr, A. Leader, F.W.K. Kan, S. Bjarnason, R. Vincent and D. Blakey

Trichloroethylene (TCE) is a volatile chemical used extensively in the automotive and metal industries for vapour degreasing and cold cleaning of metal parts. It has been estimated that 3.5 million workers are exposed to TCE (NTP, 1988).

We have used a mouse model to identify the toxic effects of TCE in the male reproductive system. Mice were exposed to TCE (1000 ppm) by inhalation for 6 h/day, 5 days/week for 1-4 weeks. Exposure after the first week was interspersed by a two-day "weekend". TCE was detected in blood minutes after exposure, and the levels were dependent on the amounts given (250, 500 and 1000 ppm). The products of biological processing (trichloroacetic acid, trichloroethanol) were also identified in blood and urine as well as in liver and testis. Chloral, another product formed from TCE, was found at higher levels in the epididymis than in the testis. Chloral was produced by the enzyme CYP2E1, which was detected at higher levels in epididymis than in testis. These results suggested that chloral formation is related to TCE-induced toxic damage.

TCE exposure of mice caused damage to the epithelial cells lining the epididymis; these cells were seen sloughing off into the lumen at 4 weeks. Observation at high resolution showed that sperm residing in the epididymis were damaged. Also, cell membranes and structures within the cell were disrupted. These alterations were seen as early as 1 week after TCE exposure.

The function of sperm was studied in mice after TCE exposure. Sperm were collected from the epididymis and incubated with mature eggs to evaluate their egg binding ability. The numbers of sperm bound per egg were counted and used as indicators of their egg binding capacity. Sperm-egg binding decreased by 56, 20 and 38% after 2, 4 and 6 weeks of TCE exposure, respectively.

To determine relevance of the mouse findings to humans, 10 auto mechanics that use TCE on a routine basis were recruited into our study. Their semen parameters were evaluated according to World Health Organization criteria as well as by the acrosome reaction (ability to shed a cap overlying the sperm head). Only one subject had normal semen parameters. The remaining subjects had one or more abnormalities in sperm parameters. One subject had relatively normal sperm parameters but sperm-egg binding was only about 25% of the control level. These results suggested that the sperm in these subjects are defective and may be associated among other factors with TCE exposure.

We also studied sperm development in mice exposed to TCE. Seven distinct subpopulations of spermatogenic cells were isolated and their levels of maturity examined. Exposure for 3 days caused cells in initial stages of development to be reduced by about 45% of the control level. After 2 weeks of exposure, there was a rebound and cell numbers increased and returned to normal levels after 4 weeks. Also, testosterone production was slightly reduced after 3 days but was relatively normal after 4 weeks. These results indicated the effects of TCE on sperm development were transient.

Taken together, our data show that TCE produces harmful effects to the male reproductive system, leading possibly to reduced fertility.

# Thymic fetal organ culture as an organ assay for EDCs

Bernier, J., Cyr, D., Fournier, M., Potworowski, E.F.

In the past 20 years, there has been a grow ing awareness in industrial, academic, regulatory and public sectors on the health risks associated with the exposure to persistent toxic chemicals, especially those interacting with the endocrine system. These pollutants, collectively referred to as endocrine disruptors, include compounds from various chemical classes (heavy metals, polychlorinated biphenyls, dioxins, bisphenols, etc). There is an impressive body of evidence showing that these chemicals can severely impact health when exposure occurs, even at low doses, at sensitive period of embryonic development. It is a general consensus that these compounds may cause, directly or indirectly, detrimental effects on four main physiological targets: endocrine, reproductive, nervous and immune systems. The question of the critical period of exposure is particularly important in trying to establish the immunotoxic potential of a test compound that may act as endocrine disruptor during the development of the immune system since all of the present testing strategies have been designed to assess the immunotoxicity of a test compound when both exposure and toxicity occur during adulthood.

In the program we want to establish the sensitivity of FTOC towards exposure to endocrine disruptors and relevant mixtures of environmental contaminants in order to validate the use of FTOC as an organ screening assay for putative endocrine disruptors. To validate this model, we propose to use a combination of *in vitro* and *in vivo* exposure protocols.

First step was to assess gender difference and effects of hormonal modulation on T-cell differentiation. This part of the research represents a very important step in the validation of this organ assay for endocrine disruptors. First, we were able to demonstrate, in the developing thymus, an absence of differences between sexes in the expression of hormonal receptors as well as in the proportion of T-cell subpopulations. This finding which represents a biological important information, allows a methodological simplification by making facultative the sexing of embryo prior to the removal of thymuses. Moreover, this part of the research program was essential to clearly demonstrate the sensitivity of the T-cell differentiation process to hormonal variation, at least for estrogens and thyroid hormones. We are actually completing the study of the effects of hormones addition for adhesion molecules and hormone receptors, to make a complete story. We are also studying the biological significance of these thymocytes subpopulation modifications in terms of effects on immune competence of the resulting pups and adults. This work is being done using a drug treatment. It is very interesting to point out the difference in the pattern of effects recorded for estrogen as compared to thyroid hormones. It is also important to point out the selectivity of the targeted subpopopulation affected by hormone variation as compared to the effects of metals, affecting several subpopulations of thymocytes. These findings suggest strongly that the model can produce clear distinction between endocrine modulation and more classical general cytotoxicity. This assay can therefore be added to the battery of test used to assess toxicity of drugs and chemicals before their acceptance.

# Did persistent contaminants affect immune and thyroid function and reproductive development of herring gull chicks in the lower Great Lakes in the 1990s?

G.A. Fox, L. Shutt, K.A. Grasman, and F.M.A. McNabb

The Herring Gull has been used extensively as a sentinel in which to monitor concentrations and effects of contaminants in Great Lakes food chains. We examined the immune function, thyroid economy and reproductive development of Herring Gull chicks from colonies in the lower Great Lakes and a reference colony in the Bay of Fundy in relation to concentrations of a suite of persistent contaminants in their tissues. We sampled at two developmental stages, near term (pipping) embryos and 4-week old chicks (prefledglings).

We have consistently found suppression of T- cell-mediated immunity and alterations in antibody production upon standardized challenge in gull chicks from Great Lakes sites heavily contaminated with polychlorinated biphenyls (PCBs). These functional affects were accompanied atrophy of the thymus and alterations in bursal cell numbers and viability.

Consistent with thyroid gland enlargement (goitre) observed in adult Herring Gulls from the Great Lakes in the 1970s and 1980s, we found depressed plasma thyroxine concentrations and goitre in prefledglings from high PCB sites in all years, indicative of hypothyroidism. Although pipped embryos showed evidence of thyroid gland hormone depletion, their circulating thyroxine concentrations only suggested hypothyroidism in 2000. Over all, our results are consistent with environmental PCBs altering thyroid function during embryonic and early post hatching development.

Nodules of residual ovarian primordial germ cells were found at a high incidence in the testes of newly hatched chicks in one Lake Ontario colony in the mid-1970s. In our current studies, such nodules and other gonadal abnormalities were found more frequently in the testes of pipped embryos at more contaminated Great Lakes colonies than the reference site, and these nodules persisted in prefledglings. We are currently measuring the concentrations of the estrogen-inducible protein vitellogenin in plasma of prefledglings as a functional indicator of exposure to nonylphenol and other estrogenic substances.

PCBs and DDE were the most abundant residues in eggs, yolk sacs of pipping embryos, and livers of prefledglings. In livers of prefledglings, polycyclic aromatic hydrocarbons (PAHs), mercury, cadmium, selenium, and lead were present in very low amounts. Unexpectedly, nonylphenol concentrations were similar to those of DDE. These data are consistent with our hypothesis that PCBs are the contaminant most likely to be associated with the immune and thyroid effects, but DDE and nonylphenol may influence the development of the reproductive tract. It is apparent that current concentrations of contaminants in the food chains of the lower Great Lakes are sufficient to affect immune and thyroid function and reproductive development of this resident, fish-eating bird. Our findings will help clarify the relationship between these endocrine-mediated health effects and various contaminants, and have implications for other fish-eating wildlife populations as well as vulnerable human subpopulations in the Great Lakes basin.

# Effects of a synthetic estrogen on aquatic populations: a whole lake experiment at the experimental lakes area

Karen Kidd

Considerable evidence exists that aquatic organisms are being exposed to and impacted by a wide range of compounds that mimic hormones. Fish exposed to these compounds often exhibit an array of responses including depressed hormone levels and reduced gonad size, and males have become feminized through the development of egg proteins and eggs. Recent studies have shown elevated egg protein precursors, vitellogenin, in male fish downstream of sewage treatment plants. Natural and synthetic (birth control and hormone replacement therapy) estrogens excreted by women are found in the aquatic environment because they are not being completely broken down in the sewage treatment plant process. While sewage effluents contain a variety of substances, it is believed that the estrogens are mainly responsible for the impacts seen in fish downstream of the plants. However, it is not known whether these potent hormone mimics are affecting the ability of fish to reproduce and sustain their populations. The following experiment was designed to address this unknown.

We are conducting a whole-lake addition experiment at the Experimental Lakes Area (www.umanitoba.ca/institutes/fisheries), northwestern Ontario to determine the short- and long-term impacts of a synthetic estrogen on well-defined aquatic populations. The lake chosen for the experiment, Lake 260, has long-term records on the fish populations, is 34 hectares in area, and has a maximum depth of 14 m. In 1999 and 2000, we collected background information on population size, reproduction (e.g. egg size and stage of maturation), and several biochemical measures (such as vitellogenin and hormones) for fish in this lake and in several reference lakes. We also collected and analysed background samples of algae, bacteria, zooplankton, tadpoles, leeches, and other bottom-dwelling invertebrates in order to determine whether hormone mimics can directly affect the organisms that fish rely upon for food. In the summer 2001, we added the synthetic estrogen, ethynylestradiol (used in birth control pills), continuously to the surface waters of Lake 260 at environmentally-relevant concentrations. This estrogen is known to be effectively degraded by bacteria present in the lake, and water concentrations rapidly decrease in the lake once additions are discontinued.

The research conducted in 1999 and 2000 on Lake 260 and the reference lakes was repeated in 2001 to look for effects of this estrogen on the fish and lower-trophic-level biota. To date, we are finding high concentrations of vitellogenin in the male and female fish from the treated lake, indicating a disruption of their normal hormone system function. We have also observed reduced hatching success for green frog eggs exposed to the estrogen, but no effects on bacterial and algal communities. Many of the samples collected in 2001 and the data on fish populations are currently being examined for initial effects on individuals and populations. For several years following the estrogen additions, we will continue to monitor the aquatic populations in Lake 260 to determine whether the impacts are short or long term, and to assess the timing and magnitude of recovery. Results will be critical in determining whether hormone mimics affect the ability of aquatic organisms to reproduce and sustain their population size. In addition, it will provide a better understanding of the risks that these compounds pose to wildlife.

# Detailed endocrine assessments in wild fish and characterization of responsible EDCs at pulp mill sites

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Impairment of reproductive fitness in fish exposed to pulp and paper mill effluents represents one of the most well documented cases of wildlife endocrine disruption in Canada. Following a regulatory package in 1993, a number of improvements have been seen in the effluents released from pulp and paper mills, however these regulations were not designed to remove the chronic reproductive effects demonstrated in wild fish populations. While some partial recovery in reproductive function has been seen at some sites, alterations in steroid levels and gonadal development are still present. Conclusions from the Northern River Basins Study in the mid 1990's suggested that similar reproductive effects are also present in wild fish downstream of some of the most modern mills in Canada. It was recommended at that time that a thorough endocrine evaluation of these fish be made. The objectives of this project were threefold; 1) to assess reproductive function in wild fish within the Northern Rivers Basin downstream of three of the most modern mills in Canada to determine whether reproductive effects were present, 2) an *in vitro* effluent screening study of various pulp mills to determine whether endocrine active compounds were present in effluents of different pulping and treatment technologies, and 3) an investigation of the endocrine active chemicals accumulated by fish using a number of *in vitro* assays and to compare these compounds to the wild fish responses demonstrated in objective 1.

Wild fish were collected at a number of locations both upstream and downstream of effluent inputs on two different river systems within the Northern River Basins, Alberta. Samples were collected and analyzed for all of the existing reproductive endocrine endpoints by the various participating laboratories. Comparisons to date between upstream reference fish with the downstream effluent exposed fish have demonstrated some alterations in reproductive function, especially downstream of a municipal sewage effluent input. Effluent samples have also been collected from 10 different pulp and paper mills across Canada and were returned to the laboratory in Burlington. Effluent extracts are being run using a number of laboratory assays and preliminary results indicate that all effluent samples contain compounds that have endocrine activity. Comparisons are now being made between different mill process types and treatment strategies to determine whether some conclusions can be made as to which process and/or treatment are most effective. Fish caging studies have also been conducted at two mills that have previously demonstrated reproductive effects in wild fish. Livers from these fish were pooled and extracted, then split into different fractions based on their lipid solubility. These fractions are now being tested using a number of endocrine assays in attempts to identify the responsible fractions and possible chemicals involved. Following the completion of analyses for the three objectives, results will be combined in hopes of making some concrete conclusions with respect to the effects of different processes and treatment strategies on endocrine function in fish populations.

# Effects of endocrine disruption on fish reproduction

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Endocrine disruptor substances (EDS) are chemicals that are capable of altering the reproductive biology of fish, wildlife and humans. Techniques have been developed to detect whether fish and wildlife have been exposed to EDS. However, little work has been done to determine whether EDS exposure can affect the reproductive capability of fish and wildlife. If individual organisms cannot reproduce, there is significant risk of population extinction; especially if EDS exposure is combined with other stressors such as habitat loss, climate change and overfishing/overhunting. The focus of this project is to determine the effects of exposure to EDS on the reproductive potential of fish through both laboratory and field investigations. In field studies conducted in 1999 and 2000, white perch were collected from several sites in Lake Ontario and Lake Erie, including Coote's Paradise in Hamilton Harbour, the Bay of Quinte, Lake St. Clair, Western Lake Erie and the Detroit River. "Inter-sex" of the gonads was observed in 18% to 80% of male white perch collected from all of these areas. The intersex condition can be observed microscopically as female ovary tissue distributed throughout the male testes. It is not known whether this intersex condition can affect the ability of male fish to spawn. To answer this question, we conducted a laboratory experiment with an aquarium fish, the Japanese medaka in which we exposed the fish to the synthetic estrogen used in birth control pills, ethinylestradiol. This compound has been found at part per trillion concentrations in the effluents of sewage treatment plants that discharge into the Great Lakes. Among male medaka exposed to ethinylestradiol at concentrations of 10 parts per trillion, reproduction with unexposed females was almost completely stopped. Of these male fish, over half had intersex gonads, which leads to speculation that males with intersex are incapable of reproducing. However, in this experiment, both the intersex males and the normal males were equally incapable of spawning with females. It appears that other factors such as alterations to mating behaviour may be responsible for the poor reproductive performance of male fish exposed to estrogens. These results are important because they show that fish reproduction is impaired by exposure to estrogenic EDS. The commercial and sport fisheries in Lake Ontario and Lake Erie are multi-million dollar industries. If fish in the lower Great Lakes cannot reproduce because of exposure to EDS, this would obviously negatively impact the Canadian economy.

# Effect of endocrine disrupting chemicals in polar bears

Ross Norstrom, Ian Stirling, Nick Lunn, Stelvio Bandiera, Robert Burk, Hans-J. Larsen, Janneche Skaare, Andrew Derocher, Oystein Wiig

Polar bears are at the top of the Arctic food chain, because their diet consists almost entirely of seals. Because polar bears may not eat for very long periods of time, they need to put on a lot of fat, so they prefer to eat seal blubber. Persistent toxic chemicals like PCBs and DDTs accumulate in the blubber, so the polar bear is exposed to high concentrations of these chemicals. It is considered to be the Arctic animal most at risk from the effects of persistent toxic chemicals. In addition to the chemicals themselves, some of the breakdown products (metabolites) in seal and the bear also accumulate, and may contribute significantly to toxic effects. This proposal addressed three areas of concern for toxicity.

One is the effect on the immune system, which is considered to be one of most sensitive to the effects of halogenated organic compounds. PCBs have usually been implicated. Other compound classes need to be considered, especially chlordane compounds and metabolites in polar bears. Norwegian studies have shown that bears from the European Arctic, which are more contaminated than in Canada, have immune systems that are suppressed. This is thought to be due to PCBs, which are significantly higher in this area than in Canada. In order to see if this effect is still found at lower exposure to PCBs, the study was repeated in Churchill, MB in the summer of 1999. There is a clear indication from the results from Svalbard that IgG was negatively correlated with total PCB concentrations in blood, indicating a possible immunotoxic effect. Thus, the effect should prove to be less significant in Canadian polar bears. When the data are completely analyzed, the immunotoxicology experiments will contribute to a better understanding of the immune system and hormone function in polar bears with high pollution levels, compared to polar bears with lower pollution levels, and thereby provide insight into the effects of pollution. Norwegian scientists have developed both reagents and methods to measure polar bear immune function, and provide a monitoring method for assessing population status over time. Immune pathological studies of PCB effects on the polar bear immune system, immune response ability, and infection resistance are important bases for development of good monitoring methods.

The second study was designed to look at whether there is an effect of PCBs on testosterone metabolism in male bears. If testosterone is metabolized more quickly, or in different ways, in highly PCB-exposed bears, it could affect reproduction. This study also looked at how various types of PCBs are metabolized by bears. Some correlations between the different type of metabolite formation exposure to particular varieties of PCBs was found, indicating the possible effect of PCBs on endocrine function in polar bears. There is evidence that testosterone levels are lower in blood of male bears with high PCBs. Further research on female polar bears is required.

The third part of the proposal was to study the effect of persistent PCB metabolites (called hydroxy-PCBs) on transport of thyroxine and vitamin A in blood, which is accomplished by binding to a special protein in blood. Some hydroxy-PCBs have a chemical structure very similar to thyroxine (the main form of thyroid hormone transported in blood), and displace it from the protein, prevent the binding of vitamin A, thus lowering the blood concentration of both. Thyroxine and vitamin A are important for growth and maintenance of healthy tissues. It was found that the binding capacity of plasma for thyroxine was negatively correlated with hydroxy-PCB concentrations, whereas the vitamin A content of plasma was negatively correlated with total PCBs. This suggests that hydroxy-PCBs may interfere with thyroid hormone transport, whereas PCB concentrations affect vitamin A concentrations by a different mechanism. However, a combination of the two effects may influence growth and development.

# Impact of endocrine disrupting compounds on amphibian health in agricultural ecosystems

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The overall purpose of the research conducted in this TSRI project was to examine the effect of environmental contaminants on amphibians inhabiting agricultural areas. More specifically, we were interested in whether contaminants used in agriculture (including pesticides and fertilizers etc.) are causing negative impacts on amphibians living in these areas. Agricultural contaminants that interfere with the normal functioning of the endocrine system of these animals, the so-called Endocrine Disrupting Compounds, or EDCs, were of particular interest.

We accomplished this by assessing the "health status" of amphibians inhabiting agricultural areas and by exposing amphibians in the laboratory to contaminants that were found in the agricultural areas. Contaminant effects were assessed by examining measures such as acute toxicity, hormonal and physiological status, physical and developmental abnormalities, immune function, sexual development and differentiation etc.

Results to date suggest that amphibians can be adversely affected by their exposure to agrochemicals. For instance, thyroid hormones are quite important to amphibians because they are directly involved with the change of larval amphibians to the adult body form at metamorphosis. Our work suggests that there may be alterations of the levels of thyroid hormones in the blood of animals exposed to agricultural contaminants. This may have important consequences on the ability of some animals to successfully complete metamorphosis, or it may influence the length of the larval period, which may cause problems in areas where the animals must complete metamorphosis before the temporary waterbodies they are breeding in dry up. Another adverse effect we observed was a decrease in the functioning of the animals' immune system as a result of exposure to pesticides. This might have important implications in terms of the animals' ability to ward off disease, and may help explain why there is an increasing incidence of disease in amphibians. In one other example, our results also support the assertion that agricultural contaminants cause an increased incidence of animals that have severe developmental abnormalities; for instance, we observed problems with sexual development in the laboratory, and a much higher incidence of developmental deformities in animals that are breeding in agricultural ponds as opposed to animals that breed in nearby less contaminated areas.

In certain areas of intensive agriculture, farm ponds may be the only habitat available for amphibians to live and/or breed in. Our results show that some of the environmental contaminants that are reaching these ponds can have adverse effects on amphibians. Because it is now widely accepted that there is a decline of amphibian populations around the world, it is important to identify all the potential causes of these declines. We also hope to determine methods by which the exposure of the animals could be diminished. Finally, it is also important to find critical periods during the life cycle of amphibians when these animals should not be exposed to agricultural chemicals.

# Involvement of P-Glycoprotein in Environmental Endocrine Disruption

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This study examined the functioning of the P-glycoprotein drug pump that is present in the kidney, liver and gastro-intestinal tract, which has been suggested as a mechanism which protects these organs from environmental contaminants.

# Exposure and effects of endocrine disrupting substances associated with intensive agricultural practices

Mark Servos, Neil Burgess, Kent Burnison, Ken Doe, Bill Ernst, Mark Hewitt, Ed Topp, Mark McMaster, Chris Metcalfe, Jim Sherry, Kevin Teather, Glen Van Der Kraak

It has been well documented that aquatic ecosystems can be exposed to chemicals through a variety of intensive agricultural practices. A wide range of substances are applied to agricultural fields that have been shown to contain chemicals that can enter adjacent waterways through runoff or direct releases. Many of these chemicals have the potential to disrupt endocrine function, and therefore alter the reproduction and development of sensitive organisms in these environments. The major areas of concern associated with these activities and chemicals include:

a) A broad array of pesticides that are widely used and several have been identified as potential endocrine disrupting substances (EDS); although pesticides have been highly regulated and controlled, the emergence of endocrine disruption issue has raised new concerns.

b) Animal wastes (manures) are applied to fields as fertilizers and as a means of waste management and these wastes contain large amounts of potential EDS (e.g.,17β-estradiol); animal production facilities in Canada are rapidly increasing in size and density.

c) Sewage sludges are commonly used as an amendment to soils and there is very little known about the mobility and fate of the EDS they contain; effluents from Canadian sewage treatment plants have been documented to contain EDS including alkylphenols, as well as natural and synthetic estrogens.

This project has investigated and documented the exposure and effects associated with these types of substances arising from intensive agricultural practices across Canada. Studies conducted in PEI and New Brunswick documented exposure of streams to a variety of pesticides, although few direct impacts on endocrine function were detected in either fish or amphibians. Studies at farms in southern Ontario were able to isolate and identify a number of endocrine active compounds in animal wastes, including natural estrogens and metabolites of phytoestrogens (natural plant products). These compounds were found to move through the soils and tile drains and enter adjacent streams immediately after manure applications and rainfall events. The biological responses of aquatic organisms in these streams were assessed during these short term pulses of exposure. Components of sewage and sewage sludges that are routinely applied to fields were also found to be estrogenic in laboratory systems. These compounds found in sample from across the country include contaminants such as alkyphenols, bisphenol-A, pharmaceuticals and natural estrogens. Laboratory testing has evaluated the potential estrogenic potency and dose response of several of these chemicals so that risk assessment and risk management approaches can be developed. A variety of estrogenic compounds were identified in agricultural systems and they appear to be capable of moving off of the fields and into adjacent waterways. Under best management practices the extent and duration of the exposure is limited and the potential impact to the environment minimized. However, the ecological significance of these exposures, especially during critical development of sensitive organisms, remains a concern.

# Whole animal laboratory testing methods for endocrine disrupting chemicals in fish

Glen Van Der Kraak, Simon Courtenay, Robert Devlin, Deb MacLatchy, Chris Metcalfe, and Joanne Parrott

Numerous studies have shown that aspects of growth, reproduction and development of fish are sensitive to the effects of endocrine disrupting chemicals including those found in the Canadian environment. Consequently, there is intense interest internationally in the development, validation, and standardization of laboratory testing protocols using fish in order to determine the potential impacts of pure compounds and complex effluent mixtures with endocrine-active properties. This project involves evaluating a series of test conditions and endpoints that can be used to define the responses of selected fish species to endocrine disrupting chemicals at various times throughout development.

The development of both short term and long term test methods involved whole animal exposures to a suite of standard compounds with known endocrine disrupting potential. These compounds included a potent estrogen agonist (ethinylestradiol), a strong estrogen antagonist (ZM-189,154), as well as an androgen agonist (methyl testosterone) and an anti-androgen (cyproterone acetate). Specifically, the test conditions examined included: 1) an early development test encompassing egg development through hatching and swim-up, 2) a gonadal recrudescence test evaluating gonadal development, 3) an adult reproduction test evaluating gonadal maturation and reproductive success, and 4) a full life-cycle test encompassing embryo hatching through reproductive development and continuing to the viability of the $F_1$ generation. The research utilizes both native species (fathead minnows, mummichog, Chinook salmon) and a laboratory model species (Japanese medaka). Currently, these tests are being used to assess environmental samples collected at pulp mill and sewage treatment facilities for reproductive and developmental effects on fish.

These studies have shown that there are marked differences in the responses and sensitivity of fish to endocrine disrupting chemicals throughout development. Immature and sexually mature fish exposed to high levels of model test compounds for as little as 7-days lead to significant changes in plasma sex steroid levels. In longer-term tests, fertility was shown to be much more sensitive endpoints with response thresholds often reduced by one or two orders of magnitude. In other studies, early stages of development were shown to be particularly vulnerable to the effects of ethinylestradiol, pulp mill effluent and sewage treatment effluent. In these cases, exposure during the period of sexual differentiation led to changes in gonadal development, including phenotypic sex reversal that was manifest later in life. An overall conclusion of these studies was that fish tested were more susceptible to compounds with an estrogenic mode of action compared with androgenic compounds.

Collectively, these studies have contributed to the development and validation of a suite of whole animal tests that can be applied in both freshwater and marine conditions to evaluate the responses of fish to endocrine disrupting chemicals.

# Urban Air

Air pollutants have an effect on the quality of urban air. Exposure to airborne pollutants has been linked to a variety of respiratory and cardiac health effects. Since its launch in 1998, the TSRI has allocated over $7.2 million in funding 20 research projects in the area of Urban Air Pollutants.

In fiscal years 1999-2000 and 2000-2001, applicants were asked to accelerate research to characterize urban air quality as well as human exposure to airborne pollutants and their effects on respiratory and cardiac health.

### In fiscal years 1999-2000 and 2000-2001, the priority knowledge needs were:

- To identify the critical components and sources of the air pollution mixes that affect ecosystem and human health responsible for cardio-respiratory disease;
- To characterize the dose-response relationships for health and environmental effects in order to determine the risks from airborne pollutants;
- To determine the biological mechanisms by which air pollution (particulate matter, chemicals and biologicals) induces cardio-respiratory diseases; and
- To develop an improved understanding of the chemical constituents and sources of airborne particulate matter (PM 2.5 and PM 10) that will facilitate the implementation of improved models to identify hot spots and predict episodes of increased health risk to susceptible populations, especially children.

### In fiscal year 2001-2002:

There were no call for proposals in fiscal year 2001-2002, therefore, no knowledge needs were developed for any of the priority research areas. However, there were still ongoing research projects which were previously approved in the 1999-2000 fiscal year.

# Chemical sources of toxic polycyclic aromatic hydrocarbon derivatives

Jonathan Abbatt
University of Toronto

It is well known that a class of molecules called polycyclic aromatic hydrocarbons is formed in combustion sources, such as in an internal combustion engine of an automobile or in a fossil-fuel-fired power plant. The larger fraction of these compounds are sufficiently non volatile that they condense onto aerosol particles in the atmosphere, such as combustion generated soot particles. It has also been known for a number of years that many polycyclic aromatic hydrocarbons are toxic species being both mutagenic and carcinogenic. One fate of these compounds in the atmosphere is that they can react with gas-phase pollutants, such as ozone and nitrogen dioxide, to yield chemical derivatives which are either less toxic or, in some cases, considerably more toxic than their precursors. Although this chemistry has been studied by a number of research groups, the detailed mechanism by which these chemical transformations occur is still not well understood. Indeed, it is not currently possible to predict whether polycyclic aromatic hydrocarbons that are emitted by an urban combustion source will be reacted away via these gas-particle interactions or whether they will suffer another fate, such as photodegradation or loss to the ground surface via particle deposition.

The overall goals of this project were to perform laboratory experiments of the chemical fates of polycyclic aromatic hydrocarbons via a mechanism where a gas phase species interacts with a condensed form of the molecule. Only with fundamental chemical information about the rates and mechanisms of these transformations can useful assessments of the fates of these species and of the production of toxic products be made. We paid particular attention to two common polycyclic aromatic hydrocarbons, pyrene and benzo[a]pyrene, and to the gas-phase reactants nitrogen dioxide and ozone. Experiments were carried out with both pure forms of the polycyclic aromatic hydrocarbons and on laboratory-generated soot surfaces which contained high levels of polycyclic aromatic hydrocarbons. Experiments were conducted primarily in a static mode, where the polycyclic aromatic hydrocarbon/soot surfaces were exposed to the gases for a known period of time and then chemically anaylzed using chromatographic methods to see the extent of reaction. The results we obtained were the following: 1. The reactions proceeded faster at higher relative humidities than in dry conditions, 2. The reactions proceed much faster on acidic surfaces than on neutral ones, 3. The reaction mechanism does not involve a simple collision of a gas-phase reagent with the surface, 4. The reaction proceeds very rapidly on the surface of the soot substrate but polycyclic aromatic hydrocarbons contained on the inside of the film are not reactive. Together, these results imply that the best manner to study these transformations is via an approach where gases are exposed to suspended soot particles. We are currently developing such an approach at the University of Toronto using the most state-of-the-art analytical methods.

# Contribution to the identification of airborne particles in Quebec City

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**Purpose:** The objective of the project was to sample airborne particles in the Quebec City area. Samples of airborne particles were collected, sorted according to size (by size fractions between 0.1 and 10 µm in diameter) and analyzed to determine their general chemical composition. The chemical composition of the particles was studied as a function of their size.

**What we did:** We collected airborne particles in central Quebec City for a number of weeks and these samples were then analyzed using various methods. The project examined the metal content of the particles and looked at priority toxic substances, as well as the concentration of potentially harmful compounds on particle surfaces, which is a factor that increases bioavailability and toxicity. Finally, in a major, innovative part of the project, we attempted to show that the surface of airborne particles acts as a reservoir, accumulating and concentrating certain ambient gases. We focused particularly on nitrogen (N2), oxygen (O2), carbon dioxide (CO2) and water vapour.

**Results:** We were unable to detect any heavy metals in or on the airborne particles collected. We showed that airborne particles adsorb much more CO2 than any other gas present in the atmosphere, such as nitrogen or oxygen. This leads us to believe that other potentially poisonous gases or vapours, could become attached to these airborne particles during their production or while they are in the atmosphere.

Although we were unable to find such gases or vapours on the airborne particles we observed, we still showed that it was possible to reveal the presence of adsorbed gases on the surface of the particles.

**Importance of the study:** Although this study did not produce many immediate results, it has opened the door to further research. The presence of harmful substances on the surfaces of the airborne particles significantly increases the chances of their entering the human body through the pulmonary alveoli and, from there, through the blood. This phenomenon is called bioavailability. Much larger studies must be carried out in order to effectively reveal the presence of potentially or actually harmful gases and vapours.

# Impact of wood burning on population exposure to the pollutants emitted

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A recent study shows that approximately one-third of Quebecers heat with wood. The ice storm in 1998 also prompted a number of Quebecers to install wood heating systems as a backup. Wood burning in stoves and fireplaces can be a significant source of pollutants. Some have even suggested that the use of wood stoves and fireplaces may be a major source of particulates. The 1995 National Pollutant Release Inventory indicates that wood heating is the third largest source of emissions of fine particulates, producing 22% of all such emissions annually. A recent report by the *Regroupement montréalais pour la qualité de l'air* on the impacts of air pollution on health and the environment also identified wood heating as one of the major sources of air pollution in the greater Montreal area; according to the report, it is responsible for 53% of total suspended particulate (TSP) emissions and 26% of volatile organic compound (VOC) emissions, such as formaldehyde. Some of these pollutants have harmful health effects, particularly in more sensitive individuals, including asthmatics, young children, and seniors.

The main purpose of this feasibility study, conducted from January to March 2000, was to determine whether it is possible to differentiate users of wood burning systems from non-users in terms of exposure to various pollutants (fine particulates, total particulate matter, aldehydes, polycyclic aromatic hydrocarbons (PAH), carbon monoxide). To that end, two zones were identified on Montreal Island: one with a high number of wood burning system users, and the other with a low number. In each of the two zones, samples were taken in 20 houses during two 24-hour periods (24 hours on the weekend and 24 hours during the week), both inside and outside. Urine samples were also collected in order to assess exposure to PAHs. Questionnaires and logbooks were given to participants to obtain information on factors likely to have an effect of these pollutants.

Participation was excellent; no participants dropped out of the project. In terms of the overall results, formaldehyde and naphthols stand out. Higher levels of formaldehyde in inside air were observed in the area with a high number of users. The concentrations of naphthols in urine were much higher in users of wood burning systems than in non-users, even when smoking was taken into account.

Limitations were also identified, as is normal for a feasibility study. We note, in particular, that a further similar study to correct such difficulties would require greater investments in terms of sampling equipment and analytic development. This study confirmed that it is possible to distinguish users from non-users for this particular source of exposure. However, it is important to emphasize that the results obtained are all the more interesting because the participants did not have to change their lifestyles. This paints a far more accurate picture of reality, which is essential for decision making in the field of public health.

# Health effects study with enhanced characterization of the urban air pollutant mix

Brook Jeffrey, Dann Tom, Mabury Scott, Burnett Richard

Although scientists have been producing evidence that even in Canada's relatively clean cities there are health costs due to air pollution, there are several unanswered questions regarding, exactly how air pollutants affect health, which pollutants are most responsible, where people are most exposed and where each of the pollutants comes from. The research in this study focuses on Toronto and Vancouver and is geared torwards providing answers to these questions. The main emphasis of the work has been on fine particles and their carbon content. The particles in the air of our cities are made up of many chemical components from sulphur, nitrogen, small amounts of metals like lead, nickel, zinc and iron, to large molecules associated with carbon. These carbon compounds are of keen interest. This is because they are very prevalent and are a main constituent of vehicle exhaust, especially the black carbon fraction seen in diesel exhaust. There is also some health evidence that the carbon fraction may be one of the more harmful components of fine particles.

During the study, advances were made on techniques to collect and measure carbon compounds associated with fine particles, on the selection and evaluation of urban measurement sites for determining the levels of pollutants the urban population is exposed to and on the compilation of a detailed record of fine particle and related measurements to be used to examine several policy-relevant health-effect questions in the future. This last aspect of the study awaits the collection of enough daily measurements to justify linking-up with health statistics compiled as part of other TSRI studies. This is planned to occur once three years of data are available. At the present time, two years have been collected and many preliminary analyses have been carried out. These analyses have shown that levels are considerably higher in Toronto compared to Vancouver, but that in both cities the carbon fraction of fine particles is significant all year. Advanced statistical techniques are now being applied by graduate students to relate the air pollutants to their possible sources. Detailed measurements collected in Toronto in the summer of 2000 showed that the variations in the black carbon fraction of fine particles are more similar across a range of urban locations, while the organic carbon fraction is quite a bit more variable from place to place. This suggests that only measuring total organic carbon at one location may not provide reliable enough information on population exposure, but for other fine particle compounds, like black carbon, one location is sufficient.

Application of newly developed methods of collection and analysis that were focused on the organic fraction of fine particles in Toronto have also provided us with new insights. The organic fraction has been known to be difficult to measure because it is influenced by non-particle phase components sticking to the collection filter and particle-phase components evaporating off the filter. These latter components are referred to as semi-volatile compounds. During summer 2001, a new method was tested in Toronto and Vancouver and showed that the concentration of the semi-volatile compounds can be as large as the concentration of the compounds that are only in particle phase (non-volatile). The semi-volatile fraction appears to be larger at night, suggesting that during the course of a day these compounds may switch between the gas and particle phase. Other new applications that focused on the chemical determination of the organic fraction have been tested in Toronto. These tests have shown that it is possible, with organic analysis, to improve estimates of the impact motor vehicles has on fine particle concentrations and to identify other possible sources such as cooking and tire wear. Similar methods are being applied to measurements taken in Vancouver in August 2001 during a large governernment-university air quality field study. The results of this study, which are not available yet, are expected to lead to large improvements in our understanding of the behaviour of fine particles in the Lower Fraser Valley and elsewhere, which will allow us to improve our ability to predict and control their concentrations in the future.

# The adverse cardiac effects of air pollution

Robert Dales

**Purpose:** To investigate the influence of fine particulate air pollution on the heart's rhythm.

**How the study is done:** Approximately 35 subjects with angina or previous heart attacks were studied. Once weekly, for ten weeks, the heart rhythm was monitored for one day using a Holter monitor. Once weekly, for ten weeks, air pollution levels were measured.

**Results:** The study is not yet finished but there is preliminary evidence that air pollution influences the heart's rhythm.

**Importance of the study:** Hospital admissions for heart disease are higher on days of higher air pollution. Seeing the effect of air pollution in individual subjects supports the theory that air pollution is harmful for the heart, and may explain how the air pollution causes its effects.

# Urban air toxics: source signature and health effects

Miriam Diamond

Poor air quality has been associated with poor health of people living in cities and poor environmental quality in general. In response to this issue, Environment Canada and other government agencies have implemented air emission control strategies and have monitored air quality to assess the effectiveness of these strategies. Our research looks at several aspects of the issue of urban air quality, focussing on persistent organic pollutants (POPs) that contribute to poor urban air quality.

First, we have been conducting research in support of air monitoring efforts. Traditional air quality monitors require a relatively large infrastructure and thus few air quality monitoring stations are operated in urban centres. We have investigated the possibility of using "passive" sampling devices that do not require a large infrastructure for deployment and are relatively inexpensive to use. Our results show that several types of passive sampling devices provide accurate assessments of the concentrations of POPs found in the gas phase. Our preliminary results are promising, however further work is required to assess the feasibility of using impervious surfaces, such as windows, as convenient passive samplers for gas and particle phases of POPs.

Second, we looked at differences in air quality within the Greater Toronto Area. Specifically, we looked at differences in POP concentrations from downtown Toronto to Egbert that is a rural community located 70 km north of Toronto. We found concentrations at least 10 times higher in downtown Toronto than Egbert of polychlorinated biphenyls (PCBs) that were widely used throughout the city, and polyaromatic hydrocarbons (PAH) that are emitted by vehicles and other sources that burn fossil fuels. In contrast, concentrations of some pesticides were higher at Egbert than downtown Toronto. We are also looking at difference in POP concentration with height, using passive samplers deployed at several heights on the CN Tower. Preliminary results show the highest concentrations at 900 ft elevation at which height air mixes from several kilometres around the city.

Third, we have looked at a variety of chemicals in urban air and that are deposited from urban air to surfaces, using windows as a convenient sampling surface (also used in the passive sampling study). We have found that plant waxes (e.g., from plant leaves) and fats from plant and animal sources are important contributors to the grime or organic film that accumulates on these surfaces. While we are not so concerned about these waxes and fats in terms of health effects, we are concerned with the abundance of these compounds in the urban core because of their ability to trap toxic chemicals such as PCBs and PAH. We continue to puzzle over our finding of more of these compounds from plant and animal sources in downtown Toronto than in rural Egbert, particularly their abundance in winter.

Fourth, we have studied the potential for POPs in air (gas- and particle-phases) to cause adverse health effects. We have done this using a screening-level bioassay that uses a cell line (in contrast to whole animal studies). Two assays have been run. The first assay is for the aryl hydrocarbon receptor at which dioxin-like chemicals bind tightly and can cause cancer and a wide range of subtle effects related to the endocrine system. The second is for the estrogen receptor that is directly related to the endocrine system. We have found that the complex mixture of POPs in urban air particles has the greatest biological activity at the aryl hydrocarbon receptor and lesser activity at the estrogen receptor. Of lesser activity is the complex mixture derived from the gas-phase, especially with rural air samples.

# Real-time characterization of the size, chemistry and origins of urban particulate matter

Greg J. Evans
University of Toronto

Particulate matter, such as that produced by cars and industry, is believed to be one of the main contributors to health problems associated with urban air pollution. A new state-of-the-art instrument, referred to as a Laser Ablation Mass Spectrometer, has been built in order to better understand the nature of these particles. This instrument permits the simultaneous measurement of the size and chemical composition of individual aerosol particles and provides the results instantaneously. In this study, this instrument is being used to explore how and why the concentration of particles in Toronto's air changes dramatically over just a few hours. Computer programs are also being developed to automatically distinguish different types of particles and to use this information in order to recognize their origins. Using these programs, it will eventually be possible to rapidly identify the sources that are contributing to airborne particle concentrations.

The instrument has been used to compare particles present at different times during the day, during episodes of urban smog, on weekdays and weekends, and during different seasons. The instrument was also run continuously, 24 hours a day, during some intensive campaigns: a 10-day campaign during the winter and a 30-day campaign during the summer. The composition and size of the particles observed during short-term increases in the particle concentration were examined. It was found that the chemical composition of the particles varied greatly between different episodes, even for short-term increases that were less than 24 hours apart. This instrument allowed some of the underlying reasons causing the elevated concentrations to be identified.

Epidemiological studies have related health effects to even short term increases in the concentration of particles in urban air. However, the physiologic mechanism causing these effects remains unclear. It is most likely that it is the body's response to key chemical components within the particles that is the cause of the health effects. Hence a good understanding of the composition of the particles, and how this changes during short term increases in concentration, is essential to understanding the relationship between particle concentrations and their health impact on Canada's urban population.

# A cohort study of air quality and utilization of hospital services

Murray Finkelstein, Dave Verma, Malcolm Sears, Kenneth Chapman
McMaster University and The University of Toronto.

Much of the research on the health effects of air pollution is concerned with counts of the numbers of emergency department visits or hospital admissions. The subjects of those studies are treated as anonymous and interchangeable. It is reasonable, however, to expect that members of a population would differ in their susceptibility to the adverse effects of pollution, or in their desire to utilize health services. It is thus important to take account of individual differences when assessing the health effects of air pollutants.

The objectives of this research were to explore the relationships between air pollutants and disease production or worsening, as measured by hospital admissions or visits to emergency rooms, while accounting for individual factors with data collected by chart review. We also planned to investigate individual "susceptibility" for the worsening of respiratory and heart diseases. The method involved compiling a list of study subjects drawn from family medicine practices and respiratory specialty clinics in the cities of Toronto and Hamilton, Ontario. Visits of study subjects to hospital emergency departments, and admissions to hospital, for respiratory and cardiac disorders were ascertained by comparing our lists with lists maintained by the Ontario Ministry of Health. Deaths among subjects were obtained by matching our list with the Ontario Mortality Database.

We found that death rates among the subjects attending the Hamilton respiratory clinic

depended upon where they lived within the city. We found that subjects living in poorer neighbourhoods had higher mortality rates than those living in richer neighbourhoods. This relation was observed despite the availability of universal health insurance which provides equal access to medical care despite low income. The average level of air pollutants was higher in poorer neighbourhoods than it was in richer ones. Subjects living in neighbourhoods with higher pollution levels had higher mortality rates than those living in neighbourhoods with lower pollution levels. Social and environmental factors were thus important determinants of mortality in this population.

We have ascertained over 100,000 hospitalizations among our study subjects. Analysis to explore the impact of air pollutants on the need for hospitalization is ongoing.

# Atmospheric air pollution causes heart and blood vessel disease

James C Hogg, Stephan F van Eeden

In excess of 60,000 deaths a year are attributable to atmospheric particulate air pollution ($PM_{10}$) in the United States and that the majority of these deaths are in subject with heart and lung diseases. When air quality deteriorates, there are more admissions to hospital for heart failures, heart attacks, irregular heartbeats and strokes. The biological mechanisms responsible for these associations are not well understood and are under active investigation. The purpose of our study was to determine the effect of exposure to air pollutants on heart and blood vessel diseases specifically atherosclerosis (the thickening of blood vessels responsible for heart attacks and stroke). Our hypothesis is that chronic exposure to ambient air pollution accelerates the development of atherosclerosis which leads to heart attacks and strokes.

To test our hypothesis, we have used rabbits that have a genetic defect that causes atherosclerosis naturally as they age. These animals were exposed to particulate air pollutants collected over Ottawa in 1993 fully characterized and provided to us by Health Canada. The exposure of the rabbits simulated the dose of particles received by a person over three months working in a major North American city.

The results show that exposure to ambient air particles caused inflammation in the lung that generated mediators that spilled over into the blood, activated the blood vessel lining and accelerated the development of atherosclerosis in the blood vessels supplying the heart and brain. This progression of atherosclerosis was associated with stimulation of the bone marrow to release leukocytes that participated in the development of the atherosclerotic lesions and made them vulnerable to rupture and occlusion of the blood vessel. The extent of the excess atherosclerosis in the blood vessels of the exposed group correlated with the deposition of particles in the lung.

Our results suggest that exposure to the particles present in ambient air pollution causes progression of atherosclerosis, the disease process that is responsible for most heart attacks and strokes. We speculate that these changes in blood vessels induced by exposure to air pollution, contribute to the increase in admissions to hospital for heart failures, heart attacks, irregular heartbeats and strokes.

# TSRI environmental justice and health research

Jerrett, Burnett, Kanaroglou, and Brook

Our research under the TSRI has been couched in an environmental justice framework. The focus has been to examine levels of ambient air pollution exposure in Hamilton, Ontario to determine if people of lower social status are exposed to more pollution. We have made use of data from the city's air monitoring network and the Canadian census for the period 1985-1996. These have been pooled to investigate the relationship between types of areas in the city and their levels of air pollution exposure, or more broadly, the question of environmental justice. Our research has already found that exposure levels averaged over several years display an unfair distribution. Hamilton neighbourhoods with low dwelling values, low family incomes and high unemployment rates are exposed to higher air pollution levels than wealthier areas. We are currently extending this research in the context of improving air quality in the urban region to examine if such improvements are reducing inequities over time and being equally shared by all, thus improving air quality for all groups but maintaining disparities between them. In the worst case scenario, air quality improvements may benefit the relative position of those whose air quality is already better than most, thereby raising inequities.

Zonal Time Series Linking Intra-urban Air Pollution to Mortality: Evidence from Hamilton, Canada

**Background**: Numerous time series studies have found significant associations between ambient air pollution and mortality. All of these studies have used central monitor or regional average pollution estimates that assume homogenous ambient concentrations over the entire urban area. This assumption of spatial homogeneity may be incorrect for some pollutants, leading to exposure misclassification. More important and less explored than spatial variation in pollution exposure is the location of susceptible individuals and groups who may also display intra-urban spatial patterns. Assessment of intra-urban associations based on local estimates may reduce possible bias due to exposure misclassification and failure to account for effect modifiers with local spatial patterns.

**Objective and Hypotheses**: The objective of this research is to assess the short-term association between air pollution and mortality within different zones of an industrial city. The key hypotheses are that (1) risk patterns in the zones will differ from the aggregate citywide or regional estimates, and (2) zones with lower social status will have larger pollution effects than zones with higher social status.

**Methods**: Hamilton was divided into five zones based on Thiessen polygons that used the pollution monitors as the central nodal point. Within each zone, daily counts of non-trauma mortality and pollution estimates were combined. Generalized Additive Models were run to test associations between sulfur dioxide and particulate air pollution measured by the coefficient of haze, while controlling for the confounding influences of weather, temporal trends, and serial autocorrelation in the mortality data.

**Results**: The results indicate that significant spatial variation exists in the association between pollution exposure and death among areas of Hamilton. Two areas of relatively high social status show no significant effects for particles, while the remaining zones have relative risks (RR) that are higher than the estimates derived from a citywide model. The zone with the highest pollution effects has a significant RR of 1.17 for a multi-day lag evaluated at the regional mean of pollution. This is about three times greater than the RR of 1.06 for the citywide estimate for the most significant multi-day lag. In conclusion, our results confirm the hypotheses that zonal estimates will differ from each other and from citywide estimates. The results also suggest that in areas of low social status, air pollution appears to exert a larger effect on mortality.

# Measurement and modelling of motor vehicle related air toxics along urban streets

Deniz Karman and Lisa Graham

An accurate assessment of the effects of motor vehicle related toxic emissions on urban populations is a relatively complex task, involving the quantitative characterization of the traffic, the emissions from that traffic, the exposure of individuals, and the health risks associated with that exposure. Toxic compound concentrations can show high variability over time and location, particularly in specific microenvironments such as sidewalks along busy streets, within vehicles, parking garages, near major point sources etc.

The Emissions Research and Measurement Division (ERMD) of Environment Canada and the Department of Civil and Environmental Engineering at Carleton University have collaborated in field studies aimed at detailed characterization and quantification of motor vehicle related air pollutants in urban micro-environments. The present study, carried out in the Winter and Summer of 2000, complemented and expanded a previous study in 1994.

During morning, noon and afternoon periods of high traffic and pedestrian volumes, two-hour, nose-level ambient samples were obtained for gaseous and fine particulate matter pollutants along Slater Street in Ottawa. In-vehicle gaseous samples were collected for typical long commuting trips by bus and private auto in the region during the morning and afternoon 2-hour periods. Altogether, 26 days of roadside samples and 30 days of in-vehicle samples were collected and analyzed. In parallel studies in ERMD laboratories, the emission characteristics of typical cars and buses were determined.

The air pollutant concentrations measured during the study have been compiled into an Ottawa Micro-environment Database (OMDB) which is made openly available to other researchers through the Internet (www.carleton.ca/~dkarman/OMDB.htm).

The day to day variation of pollutant concentrations observed in micro-environments is much higher than the spatial variation observed among diverse micro-environments such as the roadside on a busy downtown street, the rooftop on a parking structure, the inside of a commuter car, and the inside of a transit bus. The in-vehicle concentrations are generally the highest among these micro-environments. The in-vehicle concentrations observed during this study in Ottawa are of the same magnitude as those reported by studies in other cities but in the lower half of the range of values reported.

The database of concentrations of a large number or air pollutants that has been accumulated in these micro-environments is an important resource for health scientists who are involved in assessing the human health impact of air pollution from different sources in our daily lives.

# Population health impact of short-term exposure to urban air pollution

Krewski D, Villeneuve PJ, Burnett RT, Goldberg MS, Chen Y.

Studies have consistently demonstrated an association between current levels of air pollution and a variety of health effects, including mortality and hospitalization. Although the size of these effects is relatively small, the overall health impact is large as all individuals are exposed to air pollution to some degree. Several researchers have suggested that some segments of the population are more vulnerable to the effects of air pollution. Despite a number of air pollution studies, there remains considerable uncertainty as to which population subgroups may be more susceptible to deleterious health effects.

This current study attempts to better characterize the health risks associated with various components of air pollution among potential susceptible populations. Emphasis has been placed on estimating risks from exposure to the particulate and gaseous components of air pollution. Furthermore, efforts have been made to refine statistical models that have been used to characterize risk. This will in turn provide useful insights about differences obtained from previously published studies, with the ultimate goal of assisting in the development of Canadian-wide standards.

We have begun to evaluate whether there are susceptible populations who are more likely to suffer adverse health effects from exposure to air pollution. This question is being addressed within a group of residents, aged 65 and older, in Vancouver who has been followed over time for more than a decade. Information is available on the health care utilization patterns for each of these residents. This allows us to determine whether or not persons with underlying health conditions (e.g., asthma, cancer) are more likely to experience an adverse health outcome (e.g., death or hospitalization) on days when air pollution is high. We have recently completed analyses demonstrating that the association between exposure to fine particulate air pollution and mortality is unaffected by the socio-economic status. These analyses are being extended to hospitalization data.

Recently, we have reported on differences in estimating air pollution risks using time-series and case-crossover study designs. Results from time-series studies can be sensitive to several model assumptions, and risk estimates may contain sources of bias if cumulative exposure effects are present. The case-crossover design is an alternative method that can be used to estimate risk and has the advantage of controlling for potential confounding variables caused by fixed individual characteristics, and for time trends in the exposure data. We applied time series and case-crossover methods of analysis to assess associations between size fractionated particulate matter and hospitalization among children 6-12 years of age in Toronto. Positive associations were observed, and for the most part, relative risks estimated from the uni-directional case-crossover analysis were more pronounced relative to the bi-directional and time series analyses. Using these same data, elevated exposures to gaseous pollutants was associated with an increase in asthma hospitalization. Specifically, positive associations were found between carbon monoxide, sulfur dioxide and nitrogen dioxide, while no association was observed with ozone.

A study performed in Montreal represented the first in-depth analysis of the air pollution health effects among potentially susceptible population subgroups. Further work is required to corroborate the findings. In this regard, the future planned analyses of associations between levels of air pollution and health outcomes, particularly, among the cohort of Vancouver resident will prove valuable.

# Methods to determine biological compositions of particulate matter

David J. Miller, Jeffrey R. Brook

Recent studies by Health Canada and others have identified that fungal particles in outdoor air are associated with non-trivial percentages of emergency admissions for asthma in cities where the man-made pollution is relatively lower. One such report indicated that 10% of emergency admissions to the Children's Hospital of Eastern Ontario were associated with fungal spores in ambient air. This study is being extended to include other cities in Canada. The "Highest-Priority Research Recommendations " of the 1998 National Academy Report on particulate matter research priorities identifies the potential role of biologically-derived particulate material (bacterial toxins, spores and pollen) in understanding health effects of fine particulate matter. The panel stated that further epidemiological studies should be delayed until exposure measures for the biologicals can be improved.

The quantitative value of the current methods to determine fungal particles and pollen in outdoor air are not reliably known, are labour and skill dependent and expensive. The vision of this project was to undertake high-risk research to approach a method that might be applicable to existing PM sampling techniques. We wanted to determine whether it was possible to use the lipid signature profile method to assess fungal taxa from lipid extracts of such samples. Chemical analysis of species-specific lipids in bacteria have been used to bacteria in, for example spacecraft air, but there were no methods to do this for fungi or pollen. We wanted to develop an analytical method that was very sensitive.

The project was entirely successful in reaching the goals. Should sufficient lipid be present in an outdoor air sample, an accurate estimate of the fungal and pollen biomass can be made from the data collected. Analysis by direct HPLC electrospray MS would potentially produce data on the signature lipids present. These can be compared to reference data produced in this study on the organisms that have been associated with health effects by Health Canada and other researchers. Assuming that there are sufficient lipid amounts in the sample, an assessment of the proportions of such organisms in the sample can be reliably made. We have been able to find fungal lipid patterns in typical PM samples collected in Toronto.

When published, this will be one of a handful of studies in the literature on electrospray MS methods for lipids. This is the first report of the potential for lipid signature profiles for assessing fungal populations of any kind in environmental samples. Modern data on the lipids in the fungal taxa studied were obtained that had the useful result that even closely-related species have different lipid profiles. This technique may be useful in improving epidemiological studies of the effect of man-made particulate air pollution by including additional co-variates.

# An investigation of the PACs present in the atmospheric environment

Louis Ramaley, Robert D. Guy, and Peter D. Wentzell

Polycyclic aromatic hydrocarbons are a class of compounds containing only carbon and hydrogen atoms that are found in nature, for example in petroleum samples, and that are also produced by human activity, for example in petroleum refining and in combustion processes. These compounds are found in the atmosphere in two forms, as free molecules (referred to as vapor phase components) and attached to dust or exhaust particles (referred to as particulates). The hydrocarbons can further react to form derivatives with oxygen, nitrogen, or sulfur atoms. The hydrocarbons and their derivatives form a general class of compounds called polycyclic aromatic compounds (PACs). Some of the hydrocarbons are known to produce cancer or cause genetic mutations in humans and animals and some of the derivatives are believed to act in a similar fashion, perhaps to a greater extent than the hydrocarbons. Thus it is imperative to know not only the sources of these compounds but also the types and concentrations of the compounds produced by the various sources.

Our research has concentrated on two areas: (1) developing better methods of obtaining and analyzing samples, and (2) obtaining a set of samples which accurately represents the air quality at various locations in Halifax and Sydney, Nova Scotia. Our sampling system involves drawing air first through a fine filter to trap the particulates and then through a foam plug to trap the vapor phase components. A cyclone in the sampling stream passes to the filter only particles with diameters of less than 0.0025 mm. Particles of this size can pass into and lodge in the lungs and pose the greatest health hazard. We have developed a simple, sensitive, and reliable method (using an instrument called a high performance liquid chromatograph with fluorescence detection) for detecting and identifying the aromatic hydrocarbons on the filters and foam plugs. We have extended this method to the detection of the hydrocarbon derivatives called nitro compounds. These are considered to be even more of a hazard than the parent hydrocarbons.

Our sampling locations consist of both urban and suburban residential areas, high traffic areas, and an area very near the tar ponds (a highly polluted location) in Sydney. Preliminary results indicate that smaller molecules are trapped by the foam plug while larger PACs are found on the particulates. This is as expected, since the smaller the molecule, the greater its volatility, and the more likely it will exist in the vapor phase. Pollution is highest in the winter, when burning of oil for space heating is at its highest. There is little difference in PAC levels between urban and suburban locations in Halifax. More surprisingly, the levels of PACs in high traffic areas were not highly elevated over those in residential areas, indicating fairly rapid atmospheric mixing. One difference that was noted between high traffic and residential areas was in the type of compounds found – residential areas have higher concentrations of the larger PAC molecules (perhaps due to space heating) while smaller molecules predominate in high traffic areas. This may prove useful in identifying pollution sources. Finally, the results from Sydney were comparable to those from Halifax, suggesting that the tar ponds are not a significant source of PAC-type pollutants.

This type of information is invaluable for (1) identifying the types of pollutants in the atmospheric environment, (2) indicating the pollutant levels at various locations, (3) providing evidence as to the source of the pollutants, and (4) demonstrating the effect of weather conditions and other factors on pollution levels.

# Cardiorespiratory effects of controlled human exposures to particulate matter & ozone

Silverman F., Brook J., Liu L., Poon R., Parfett C., Vincent R., Kumarathasan P., Urch B., Tarlo S., Speck M., Corey P.

This study is designed to look at the effects of fine particles on lung function and to identify cellular and molecular biological markers of exposure. The overall objective of the study is to determine how fine particles cause adverse cardiovascular and respiratory effects. These effects have been observed in animal studies and human studies that associate outdoor pollution levels with hospital admissions, morbidity and mortality. Specifically we will look at: (1) mild asthmatics as a potential sensitive population; (2) the particle dose-response relationship; and (3) the potential effects of ozone as a co-pollutant. A multidisciplinary research team at the University of Toronto and St. Michael's Hospital in collaboration with scientists at Health Canada and Environment Canada are carrying out the investigation.

Using an ambient particle concentrator and a controlled environmental facility, volunteers (18-40 years of age and non-smokers) breathe clean air (no particles), and two different concentrations of concentrated ambient fine particles (CAPs), for 2-hrs, on three separate occasions. In addition, half of the volunteers have ozone added while breathing clean air and the two CAPs. The amount they breathe is similar to conditions during a smoggy day in Los Angeles. Lung function tests are performed, symptoms are noted, blood drawn, and lower airway and nasal passageway fluids are obtained before and after breathing clean air or CAPs. As measures of inflammation, we analyze blood, airway and nasal fluids for changes in cellularity and molecular mediators.

Preliminary analyses show no changes in lung function (including measures of lung volume and airflow), and lower airway and nasal cellularity with fine CAPs and/or ozone inhalation. We have found some interesting trends that could provide plausible links to an increased risk of cardiovascular illness following pollutant exposure. These findings include higher levels of proteins that constrict blood vessels. In addition, we have also observed an increase in proteins released by white blood cells, which are involved in inducing the production of inflammatory proteins. These inflammatory proteins play a role in the immune response & blood clotting system, as well as increasing blood viscosity.

Our quantification of the human health effects of fine CAPs and ozone in relatively healthy persons is difficult because the observed low level of response can be in some cases masked by background natural biological variation. We have made some interesting initial observations, most of which occur on a molecular level. This may be a reflection of the subtlety of minor changes in healthy people that could be more significant in the most susceptible populations- namely the elderly and those with heart and lung diseases.

Continuing to explore these minor changes is extremely important. Improving our understanding of them could lend support to the hypothesis that air pollution can lead to clinical morbidity and mortality– a significant component of air pollution policy development and appraisal.

# Controlled exposure study of the influence of urban air pollutants on the human cardiovascular system

Silverman Frances, Brook Jeffrey, Vincent Renaud, Kumarathasan Premkumari, Urch Bruce, Brook Robert, Rajagopalan Sanjay

The goals of the study were to learn more about how air pollution affects a person's health. The theory we tested in our study is that the harmful chemicals in pollution that are taken up in the lung start a chain reaction that ultimately influences how well the arteries in the heart can provide blood. Heart researchers (cardiologists) have developed a technique that involves measuring the size of the artery in a person's upper arm before and after the blood flow is temporarily stopped. In this study, human volunteers had these measurements made before and after they breathed a combination of two pollutants and before and after they breathed clean air. The volunteers did not know which of these two possible exposures they were breathing and neither did the person measuring the responses of the artery. The pollutants that the volunteers were exposed to were fine particles and ozone and the amount they breathed was similar to conditions during a smoggy day in Los Angeles. Both are common air pollutants and both are pollutants that have been linked in studies throughout the world to harmful effects such as hospital admission rates, mortality rates and even to the onset of a heart attack.

This study was carried out by a multidisciplinary research team at the University of Toronto and St. Michael's Hospital in collaboration with scientists at the University of Michigan, Health Canada and Environment Canada. From April 2000 to March 2001 twenty-five healthy volunteers were tested. They ranged in age from 18-50 (15 men and 10 women) and all were non-smokers. The measurements indicated that breathing the fine particle and ozone mixture caused the brachial artery in the upper arm to shrink by a small but measurable amount. The size of this reduction is too small to be of major concern for a healthy person, but if a person is already experiencing cardiovascular illnesses then this reduction might lead to more serious health problems. The response found in our research is consistent with other blood chemistry findings, which showed that exposure to fine particles leads to an increase in one of the proteins involved in controlling expansion and contraction of blood vessels and arteries. At the present time, our finding that the artery shrinks or constricts was not accompanied by a measurable change in blood pressure or in the ability of the artery to function. But more research is needed to examine these factors and others in more detail. If the results of this research are followed up with more detailed study and if the current consistency with other health research continues, then our work could help Canadian decision-makers set new policies to control air pollution for the protection of public health.

# Effects of air pollution on cardiac arrhythmias

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Population studies have found associations between brief increases in outdoor air pollution concentrations and measures of heart disease, including deaths from heart disease as well as heart disease hospitalizations. One possible mechanism behind this association is that exposure to air pollution increases the risk of developing an abnormal heart rhythm (cardiac arrhythmia). To investigate this hypothesis, data on patients attending the implantable cardiac defibrillator (ICD) clinics at St. Paul's Hospital in Vancouver, BC were used to see whether increases in air pollutant concentrations were associated with increased risk of cardiac arrhythmia. An ICD is a device that is surgically placed in a patient to provide an electrical shock whenever it senses a very fast heart beat in order to convert it back to a normal rhythm. The ICD keeps a record of each time it delivers a shock. This group of patients might be expected to be very sensitive to any triggers for these abnormal heart rhythms, including possibly air pollution.

Daily outdoor air pollution (breathable particles, ozone, sulphur dioxide ($SO_2$), nitrogen dioxide ($NO_2$), and carbon monoxide) and weather data were obtained for the years 1997-2000 for greater Vancouver. Air pollution concentrations were relatively low, as expected. Clinic records were abstracted for the date of each ICD shock for each patient.

There were 50 patients who lived in greater Vancouver and who experienced at least one ICD shock during the period of the study. No consistent associations between increased air pollution concentrations and increased ICD shocks were found. The analysis was then limited to the 16 patients who had at least 6 months of ICD recording and who experienced at least two ICD shocks each year in the hopes of being able to detect an association among those patients who provided the most information. In those 16 patients, an association between increased $SO_2$ and ICD shocks was detected two days after the increases in $SO_2$. No associations with any of the other pollutants were seen. Because of the sometimes dramatic differences in air pollution in different seasons, the analysis was divided into an analysis of the summer season (May - September) and the winter season (October - April). No associations between increased air pollution concentrations and increased risk of ICD shock were seen in the summer, although for several pollutants concentration increases were associated with a decreased risk of ICD shock, an association opposite in direction to that expected. In the winter, increased $SO_2$ concentrations again were seen to be associated with increased risk of ICD shock two and three days following increases in $SO_2$, but no effects of the other pollutants were seen.

These findings provide little evidence that relatively low concentrations of outdoor air pollutants contribute to the risk of cardiac arrhythmia. The findings regarding $SO_2$ are difficult to interpret. They may be chance findings. On the other hand, given the very low concentrations of $SO_2$ that were present in Vancouver, if the findings are valid, it is likely that $SO_2$ is reflecting effects of some other environmental factor, possibly another pollutant.

# Inhalation Toxicology of Air Pollutants: Pulmonary and Cardiovascular Effects

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This study used critical endpoints of pulmonary and cardiac effects, that can be related to pathobiology and pathophysiology, to determine the dose-response relationships for the interaction of inhaled ozone and particulate matter in animal models. The data will be used to develop a toxicodynamic model of pollutant interaction which will then be tested for the modulating effects of carbon monoxide and nitric oxide. These results will be used to create realistic mixtures of these pollutants which will be tested in animal models to verify the impact of biological sensitivity on the dose-response profiles.

# Forensic fingerprinting of HAPs and VOCs by C-, H-isotope biogeochemistry

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Our study examines the character of specific organic compounds extracted from filtered total suspended particulates in and around the Lower Fraser Valley (LFV), BC. The long-term objective is to identify spatial and temporal variations in both molecular and stable isotope composition of these organic compounds with the goal of understanding their sources or to provide insights into transport and/or atmospheric chemistry processes. This will provide critical information to help assess the potential type and magnitude of exposure that humans receive in such urban settings, and the associated health risks.

Samples of airborne particles are collected on quartz fibre filters at five sites throughout the LFV and at two marine locations using high volume air samplers. Each of the LFV sites was sampled on a monthly basis from March to August 2001. The samples are brought back to the Biogeochemistry Facility at UVic where they are extracted, and separated into groups of compound classes. Selected compound classes are analysed by two analytical techniques Gas Chromatograph/ Mass Spectrometry (GC/MS) and a novel Continuous Flow–Isotope Ratio Mass Spectrometry (CF-IRMS). GC/MS identifies individual compounds and determines their concentration. CF-IRMS determines the stable isotope ratio of individual compounds. Stable isotopes are useful in fingerprinting the sources of the organic matter and the processes that remove and disperse them. Carbon ($^{13}C$ to $^{12}C$ ratios) and hydrogen ($^{2}H$ to $^{1}H$ ratios) isotopes are naturally present in proportions that are diagnostic. For example, a plant during photosynthesis, will utilize more $^{12}C$ than $^{13}C$, leading to an $^{12}C$ enrichment over the original $CO_2$ carbon source. Similarly, as organic compounds are degraded by photochemical processes in the air, the $^{12}C$ reacts more rapidly than $^{13}C$, leading to enrichments of the latter. Therefore, changes in the isotope ratio (e.g., $^{13}C/^{12}C$) can detect and classify sources of the compounds and the different processes that they have experienced.

Our preliminary results, e.g., with one group of compounds that we observe (*n*-alkane hydrocarbons) indicate that the concentration of individual *n*-alkanes varies between sites and seasons. The isotope composition of these *n*-alkanes has been analysed in detail, and other compound classes are presently being studied. There are clear and diagnostic variations in the isotopic character of different compounds within the LFV. The isotopic character of the *n*-alkanes show differences in that the higher n-alkanes (those with >25 carbons) are more depleted in $^{13}C$, whereas the lower *n*-alkanes (those with <25 carbons) are enriched in $^{13}C$. This is especially evident in the summer months. The odd-numbered *n*-alkanes ($C_{21}$–$C_{31}$) are enriched in $^{13}C$, whereas the even-numbered *n*-alkanes ($C_{20}$–$C_{30}$) are more depleted in $^{13}C$. This indicates an increase in fresh, terrestrial material in the summer months, and more degraded material in the winter months. Our novel approach is proving useful to identify sources of these airborne organic compounds. To further distinguish source and sinks, our ongoing analyses include hydrogen and bulk isotope analyses, and the quantification analyses of other compounds groups (or compound classes). Ultimately, this information will allow us to estimate and better appreciate health aspects and wellness outcomes in urban air sheds.

# Respiratory inflammatory response to ozone exposures in asthmatic children & adolescents

Zimmerman B, Liu L, Vincent R, Kumarathasan P, Urch B, Tarlo S, Corey P, Silverman F

There is clear evidence of associations between daily variations in ozone air pollution & adverse health effects. Children may be particularly vulnerable to air pollution because their respiratory tracts are immature, they spend more time outdoors, are generally more active & have higher breathing rates than adults. It has been reported that breathing ozone leads to inflammation in the airway. Recent studies have suggested that ozone may intensify existing inflammation created by the allergic immune response.

This was a 1-yr study to investigate the association between ozone inhalation & respiratory inflammation. Volunteers were healthy non-smokers, 10-19 years of age, with mild asthma. For 2-hrs, including 1-hr of exercise, they breathed ozone at levels similar to a smoggy day in Los Angeles. As the main study objective, responses of 5 untreated asthmatics were examined to determine if breathing ozone increased pre-existing airway inflammation (from allergies to a house pet). Ozone responses were also examined in 4 allergic asthmatics pre-treated with an anti-inflammatory asthma drug & in 3 non-asthmatics. On a separate occasion, each volunteer also breathed clean air, as a control.

Results for tests of lung function showed no differences in mean responses between ozone & clean air, or between asthmatics (untreated & treated) and non-asthmatics. The largest decrease in lung volume for an individual after breathing ozone for 2-hrs was for an untreated asthmatic who had a 16% drop from baseline.

Cellular inflammation was assessed using a sample of airway fluid. Of specific interest was the eosinophil- a characteristic cell in asthma & allergic reactions. After breathing ozone, 4/5 untreated asthmatics had an increase in eosinophils (mean increase from 5% before exposure to 12%), with one individual increasing to 26%. Twenty-four-hrs after breathing ozone, in 2/4 volunteers, eosinophils remained elevated (mean 8%), with one individual increasing to 17%. After breathing clean air there were no changes. The two other groups did not show any changes after breathing ozone or clean air.

Of particular note was the response of a 15-yr-old untreated allergic asthmatic (not included with above results) who had hyper-responsive airways. He had 29% eosinophils in his airway fluids, well above both the normal level of 2% or less, and the level expected with mild asthma (3-7%), although he rarely reported symptoms except when exercising. After 20 min of breathing ozone, including 15 min of exercise, his lung volume fell by 50%. The ozone was stopped & he was given medication to immediately reverse the ozone-induced airway constriction. However, his eosinophils increased from 29% before exposure to a high of 49% 24-hrs following exposure. Despite his failure to recognize significant asthma symptoms, the laboratory results indicated a high level of asthma & an inability to exercise in ozone at levels similar to a smoggy day in LA.

In this study of the effects of ozone inhalation, we have shown an enhancement of inflammation in the airways of untreated allergic asthmatic children. There was however no enhancement of inflammation when allergic asthmatics were pre-treated with an anti-inflammatory asthma drug, & in non-asthmatics. Continuing this study with additional volunteers should help in the evaluation of a "safe" ozone limit in a vulnerable population & encourage public policy to reduce the levels of ozone in the air.

# Cumulative Effects

Cumulative effects is the study of the human health and ecosystem effects resulting from cumulative exposures to chemical mixtures, multiple stressors, multiple sources, and incremental impacts at various temporal, spatial and biological scales. Since its launch in 1998, the TSRI has allocated over $7.6 million in funding 23 research projects in the area of Cumulative Effects.

In fiscal years 1999-2000 and 2000-2001, applicants were asked to accelerate research needed to improve understanding of the cumulative ecosystem and human health effects of toxic substances. Applicants were also asked to address other emerging toxic substance research issues that may arise over the lifetime of the TSRI and to address toxic substance research issues that fell into more than one of the five priority research areas.

**In fiscal year 1999-2000, the priority knowledge needs were:**

- To develop tools to predict the cumulative effects of exposure to environmental mixtures of toxic substances;
- To improve our ability to monitor POPs, metals and EDCs in human tissues;
- To determine the ecosystem and human health effects associated with environmentally relevant mixtures of POPs, metals and EDCs;
- To develop biomonitoring techniques and assessment models to evaluate the cumulative impacts of toxic substances on ecosystem and human health;
- To identify the linkages among multiple assessment indicators to establish the critical thresholds of cumulative ecosystem and human health effects that result from exposure to environmental mixtures of toxic substances;
- To develop approaches to assess the cumulative effects of exposure to environmental mixtures of naturally occurring and anthropogenic toxic substances;
- To assess the cumulative effects of nutrient, temperature, dissolved oxygen interactions, and other environmental factors on the adequacy of regulations and guidelines for protection from exposure to priority metals, POPs and EDCs;
- To establish the interactive effects and mechanisms by which nutrient loadings mask the effects of contaminant stressors in the environment;
- To identify the influence of anthropogenically-influenced environmental factors such as the hydrologic cycle on contaminant exposure and effects on environmental and human health;
- To develop analytical approaches and models to trace and predict the fate and effects of multiple contaminant stressors on food webs and human health;
- To characterize the additive and incremental effects of multiple source and stressor effects from large-scale developments such as oil sand and metal mining; and
- To develop integrated assessment approaches, techniques and indices to characterize the impacts of toxic substance mixtures on microbially-based food chains.

### In fiscal year 2000-2001, the priority knowledge needs were:

- To develop analytical approaches and tools to predict and assess the cumulative ecosystem and human health effects of exposure to environmental mixtures of naturally occurring and anthropogenic toxic substances; and

- To identify and characterize emerging toxic substance research issues.

### In fiscal year 2001-2002:

There were no call for proposals in fiscal year 2001-2002, therefore, no knowledge needs were developed for any of the priority research areas. However, there were still ongoing research projects which were previously approved in the 1999-2000 fiscal year.

# Assessment of environmental risk factors for breast, colon and lung cancer in Sydney, Nova Scotia

Pierre Band, Michel Camus, Ronald Dewar, Daniel Krewski

The town of Sydney, NS, has been contaminated by coke ovens and steel foundry emissions for close to a century. In a previous study, we documented a statistically significant increased mortality for colon and breast cancer in the population of Sydney compared to the population residing in Cape Breton County excluding Sydney. The aim of this current research is to determine whether environmental factors are related to colon, breast and lung cancer in the population of Sydney; lung cancer was also included because of its association with work in coke ovens operations.

Patients with a new diagnosis of colon, lung and breast cancer and individuals without cancer (controls) are asked to provide, by means of a questionnaire, detailed information on their lifetime residential, occupational, smoking and alcohol consumption history. As well, information is collected on a number of other cancer risk factors including family history, reproductive history (age at menarche, first full term pregnancy, menopause) and diet. The data will be analyzed to verify whether, after controlling for known and suspected risk factors, the incidence of colon, lung and breast cancer is significantly higher among individuals who ever lived in Sydney compared to Cape Breton residents who never lived in Sydney, and whether this increase is associated with the number of years having lived in Sydney. Positive results would strongly suggest an association with environmental exposures to cancer causing substances.

After receiving approval from Ethics Review Committees, a project team was established at the NS Cancer Registry and at the Cape Breton Health Care Complex and procedures were initiated to obtain control subjects. A detailed questionnaire was developed and sent to cases and controls beginning on January 1st, 2000. From that date to July 31st 2001, a total of 153 breast cancer cases, 121 colon cancer cases (68 males and 53 females), 158 lung cancer cases (110 males and 48 females) and 860 controls (326 males and 534 females) were ascertained. Of these, questionnaires were received from 79 breast cancer cases, 60 colon cancer cases (35 males and 25 females), 45 lung cancer cases (34 males and 11 females) and 537 controls (197 males and 340 females).

Despite much effort, accrual for lung cancer has been low, probably because of short survival. Accrual for breast and colon cancer and for controls is on target. Statistical analysis of the data is planned to begin in January 2001.

# Risk assessment for microcystin-LR in drinking water: a rat liver tumour promotion study

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Microcystins are naturally-occurring toxins released by blue-green algae. The latter are killed by water treatment but their toxins, which are resistant to these usual treatment procedures, are then released into drinking water. Health Canada has proposed a maximum acceptable concentration (MAC) of 1.5 µg/l for total microcystin-LR in drinking water, using a Tolerable Daily Intake (TDI) of 0.04 µg/kg b.w. The latter is the level to which humans can be exposed every day for their entire life without developing toxicity. This level has been based on a threshold value (NOAEL of 40 µg/kg b.w. per day) at which no acute liver injury was observed in a 13-week mouse study. Liver cancer promotion has been observed for intraperitoneal (i.p.) injections of microcystin-LR to rats at doses lower than the level used for the TDI, as exemplified by the increased number of pre-cancerous lesions (glutathione S-transferase placental form (GST-P) positive liver foci) after eight weeks of i.p. dosing with 10 µg/kg b.w, but not with 1 µg/kg b.w, microcystin-LR (Nishiwaki-Matsushima et al., J. Cancer Res. Clin. Oncol., 118, 420-424, 1992). Thus, when taking into account the normal route of human exposure, i.e. consumption of contaminated drinking water, a question arises: Is microcystin-LR a liver cancer promoter after oral exposure? Search for a critical cellular event in the mechanism of liver tumour promotion was also undertaken.

Promotion studies were performed by using a rat model based on first, the i.p. injection of an initiator of carcinogenesis, diethylnitrosamine (DEN) at a dose of 200 mg/kg b.w., followed by treatment with the putative promoter, i.e. microcystin-LR, from week 2 to 8 and partial hepatectomy at week 3. Liver cancer promotion was assessed by comparing the number and area of GST-P positive foci in the liver of microcystin-treated rats to those in controls, i.e. given DEN alone. Older rats were used to mimic the adult age in humans since oral absorption of microcystin-LR appears to increase at older age. Promoting activity of microcystin-LR was assessed at several doses, i.e. 0, 10, 40, and 80 µg/kg b.w. given to rats by gavage. An increased number of GST-P foci was observed after oral administration of 80 µg/kg, but not at 40 µg/kg, i.e. the threshold dose used to derive the TDI; the i.p. dose of 10 µg/kg also caused a marked increase in the number of foci.

GST-P-positive foci show markedly lower gap-junctional intercellular communication. In this research, we observed specific adduct formation of microcystin-LR to only one liver protein of a molecular weight similar to that of connexin 32, a protein for which a lower level yield to reduced intercellular communication. Thus, it is believed that this event may be a good endpoint to establish a dose-response curve to determine the level without liver effect. Further research is needed to validate this finding. Overall, this work demonstrated promoting activity for low oral doses that will enable mechanistically-based risk assessment for microcystin-LR in drinking water.

# A multiple bioindicator approach to assess risk of liver cancer

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Liver tumors in wild fish may be used to detect the presence of carcinogenic chemicals in the environment that may be detrimental to ecosystem and human health. In Canada, tumors of unknown cause are observed in the liver of marine fish in industrialised and urbanised zones of the Atlantic and Pacific coasts. The association between exposure to environmental contaminants and the development of liver tumors in wild fish exposed to complex mixtures of contaminants needs to be examined.

Fish liver tumors have been frequently associated with exposure to sediments highly contaminated with polycyclic aromatic hydrocarbons (PAHs). However, high concentrations of PAHs are not always present at sites where liver tumors are observed. Other chemicals such as persistent chlorinated compounds (PCBs, dioxins, pesticides) may play an important role in the development of these lesions. The mechanisms leading to liver cancer are investigated in Atlantic tomcods collected in the St. Lawrence Estuary and Gulf and in English soles sampled along the coast of BC at sites contaminated with various levels of PAHs and persistent chlorinated compounds. Levels of chemical contaminants are measured in fish tissues in parallel to a variety of cellular responses detected by biochemical and histological methods. Particular chains of cellular events will be associated to exposure to particular groups of contaminants. Associations observed in the field will be tested experimentally in Atlantic tomcod exposed to relevant compounds such as PCB126, the most toxic congener of PCBs found in fish from the St. Lawrence Estuary.

Significant new data have already been obtained. English soles captured in Vancouver Harbour have cellular responses typical of those observed in fish exposed to PAHs with high levels of DNA adducts that may lead to increased rates of mutation and cancer. In contrast, Atlantic tomcods sampled in the St. Lawrence E. are exposed to relatively high levels of chlorinated compounds but to low levels of PAHs. They have signs of toxicity in their liver that are not typical of those associated with PAH exposure. Thus, the processes leading to the development of liver tumors differs between Atlantic tomcod from the St. Lawrence E. and English soles from Vancouver Harbour.

This project will generate new information on the role of persistent chlorinated compounds in the development of liver tumors in fish. These are of particular concern because they may be transferred to humans via consumption of aquatic organisms. The suites of bioindicators developed in this project will be applicable to international programs of environmental health monitoring.

# Alternative approaches for cumulative effects bioassessment

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This project is developing novel river aquaria that can be used to perform field bioassays that determine critical thresholds at which pulp mill and metal mining effluents affect the biology of rivers. Using these river aquaria, we are creating a new approach for assessing the cumulative effects of multiple effluents. Our focus is on evaluating the cumulative effects of complex effluents from pulp mills and metal mines because cost-effective alternatives to standard field assessments of river fish and insects are urgently needed. Currently, standard assessments cannot provide the cause and effect information that is required to understand how the potential risks posed by effluent pollution affect river plants and animals. Below we review research progress that demonstrates how the novel river bioassay approach can be used to study the impacts of effluents on fish, insects and other members of the food chain.

This project includes the evaluation of river aquaria designs for cumulative effects assessment of algae, insects, fish, and multitrophic level food chains. The work completed during Year 2 of the project included assessments at two sites in New Brunswick where we evaluated an improved river aquaria system at pulp mill and metal mine sites. The pulp mill effluent experiment evaluated consistency of river aquaria results between Years 1 and 2, tested new measures of effluent effects, and examined the effects of varying doses of effluents on river insects and fish. Studies at the metal mine site allowed a comparison of river aquaria results for fish and algal-insect food webs to study differences in effects in the food chain. In Year 3 the project is using the river aquaria approach to assess the cumulative effects of a metal mine effluent on fish populations in Ontario, and the combined effects of municipal sewage and pulp mill effluent on fish populations and river food webs in Alberta. To date, our results indicate that this river aquaria approach to cumulative effects assessment gives consistent results between years at a site. More importantly, the effects are consistent among different trophic levels in the food chain, and among individuals, populations and biological communities. The novel approach is providing new understanding of the mechanisms by which complex industrial effluents impact river biology.

# Reproductive/mammary tumorigenic effects of neonatal exposure to breast milk contaminants

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Numerous environmental contaminants are present as complex mixtures in human tissues and milk. Some include the insecticide dichlorodiphenyltrichloroethane (DDT), industrial chemicals and incineration by-products such as polychlorinated biphenyls (PCBs), dibenzodioxins (PCDDs), and dibenzofurans (PCDFs). DDT and PCBs are the most abundant human milk contaminants, whereas the PCDDs and PCDFs are sometimes referred to as the most toxic contaminants. Humans receive the highest level of exposure to these chemicals during the neonatal period (up to six months of age), particularly when infants are breast-fed. Despite this, infants derive a clear health benefit of being breast-fed. However, we do not know if there could be short or long term adverse health effects of being exposed to these chemicals during infancy. Some raised the hypothesis that it could be linked to reproductive problems, or an increase in the risk of breast cancer development. The TSRI funds allowed us to test these hypotheses. A study looking for effects of a mixture of 19 PCBs, DDT, and DDE (the major metabolite of DDT) (Mix-1) was completed, and the effects of another mixture made of 3 PCBs, 6 PCDDs, and 7 PCDFs (Mix-2) were tested. All mixtures were prepared according to the concentrations of each chemical found in human milk. Groups of baby rats were fed doses equivalent to 1X, 10X, 100X, or 1000X times the amount a human baby consumes over its first 24 days of life. Three types of experiments were performed. First, experiments were conducted to criticize, and improve the uterotrophic bioassay, one of the tests in the endocrine disrupter test battery under validation by regulatory agencies. Mix-1 had estrogenic properties *in vitro*, but it had no uterotrophic effect *in vivo*. Mix-2 is made of chemicals known to have antiestrogenic effects, and when an estrogen, 17ß-ethinyl estradiol, was tested with Mix-2, it could not decrease the uterotrophic effect of this estrogen. The sensitivity of the uterotrophic response is poor, which impair its ability to detect endocrine disrupters. Type two experiments tested for immediate toxic effects of these mixtures. Both mix were slightly toxic, they increased liver detoxication enzyme activities and liver weight, at 100X and 1000X, respectively. However, their toxicity differ given that Mix-2 reduced serum thyroxine but not Mix-1. These short term effects suggest that Mix-2 is more toxic than Mix-1, which contrast with long term endpoints, from a third type of experiments, testing for effects on reproduction and the risk of breast cancer development. None of the treatment had effects on age at puberty, estrous cyclicity, and ovarian follicular populations. Mix-2 had no effect on mammary tumor development, but it was affected in two ways by Mix-1 at 1000X. It initially delayed the development of tumors, but at the end of the experiment it increased the number of mammary lesions. Cancer development is associated with imbalance in the mechanisms of cell division and cell death. Effects of the mixtures on these mechanisms are being investigated. In all experiments, no effects were detected at the level at which human babies are exposed. A stepwise approach in these studies is required for appropriate policy decisions, and it allowed us to discriminate between the effects of Mix-1 and Mix-2. The effects of combining Mix-1 and Mix-2 together, remain to be studied.

# Assessing the cumulative impacts of oil sands derived chemical mixtures on aquatic organisms in Alberta

D. George Dixon, Andrea J. Farwell, Ralph E.H. Smith, Glen J. Van Der Kraak, Lucila E.J. Lee, Bruce M. Greenberg and Michael V. Power

Bitumen is a molasses-like mixture of oils and other materials extracted from the oil sands deposits in northern Alberta. Mining of oil sands and extraction of bitumen is an economically important industry in this region, which currently produces approximately 20 percent of Canada's oil needs. Higher production rates are expected as industry expands their existing operations and begins new developments. The extraction of bitumen from the oil sands generates large volumes of process-affected waters containing elevated levels of naphthenic acids, salinity and polycyclic aromatic hydrocarbons. Organisms living in aquatic environments located on the oil sands deposits are naturally exposed to low levels of naphthenic acids, salinity and polycyclic aromatic hydrocarbons however mining activities and reclamation practices may result in aquatic organisms being exposed to elevated levels of these chemicals. To better understand and predict the potential impacts that oil sands derived chemical mixtures will have on aquatic environments, it is important to find out how these chemicals or groups of chemicals interact with one another and how different aquatic organisms respond to these mixtures. Naphthenic acids extracted from oil sands are a complex mixture of saturated carboxylic acids that are poorly understood in terms of their chemical composition and how this mixture interacts with other chemicals, particularly salinity.

The effects of mixtures of naphthenic acids and salinity have been studied using both aquatic plants and animals. Microcosm experiments and field studies involving the collection and analyses of water chemistry and phytoplankton community composition for 30 water bodies in northern and central Alberta were conducted to determine the nature and extent of salt and naphthenic acid influences on phytoplankton communities. Photosynthetic and respiration endpoints have been utilized to determine the interactions between naphthenic acids and salinity on higher aquatic plants (*Lemna gibba*). Laboratory and field studies have also been conducted to examine the interactive effects of naphthenic acids and salinity on fish. Rainbow trout cell lines derived from gill, liver, spleen and gonads were examined for impairment in several cellular functions at various naphthenic acid and salinity concentrations and time points. Cellular-level effects of crude naphthenic acids were evaluated to develop rapid cost-effective bioassays to quantify naphthenic acid mixture toxicity and for range finding studies that could be applied to in vivo assays. Larval fathead minnow tests have been used to study interactive effects at sensitive life stages. Multiple endpoints including endocrine markers have been measured in exposed goldfish to evaluate specific mechanisms that may affect reproduction. Field sampling has indicated that sufficient data may be collected on young-of-the-year to estimate statistically valid models of the effects of contaminants and temperature on growth. The combination of toxicological endpoints examined in this study provide the information necessary to assess the cumulative impacts of oil sands mining activity on aquatic organisms so that steps can be taken to ensure the health of these organisms.

# Exposure to chlorination by-products during pregnancy and stillbirths

Linda Dodds, Will King, B. Anthony Armson, Alexander Allen

Chlorine is the most common disinfectant used in Canadian drinking water and is a cost-effective method to prevent the spread of water-borne diseases. However, it is known that chlorine reacts with natural organic matter in surface water, such as lakes and reservoirs, to produce numerous chemical by-products. In Canada, guidelines pertaining to maximum acceptable levels have been established for only the trihalomethanes, the most abundant by-product found in drinking water. Halogenated acetic acids are the next most common by-product found in chlorinated drinking water but are not routinely measured in Canada. The Canadian guideline for total trihalomethane level is currently set at 100 µg/l. Although most of the interest to date has been on the health effects related to trihalomethanes, other by-products may be implicated as health hazards.

Several of the identified chlorinated by-products have been shown to have mutagenic and possibly teratogenic properties. Recently, epidemiology studies have begun to investigate the role of chlorination by-products, specifically trihalomethanes, on adverse birth outcomes, but results are conflicting. Data from a previous study in Nova Scotia showed that women living in an area with trihalomethane levels over the Canadian guideline (100 µg/l) had a 70% increased risk of having a stillborn infant, compared to women living in areas with low levels of trihalomethanes. As well, the results indicated that the risk was especially high among stillbirths due to abruptio placentae.

The present case-control study is designed to examine, in greater detail, the relationship between exposure to chlorination by-products and stillbirths. Eligible subjects include all women who had a stillbirth (cases) and a random sample of women who had a live birth (controls) from Nova Scotia and Eastern Ontario between May 1, 1999 and December 31, 2001. Women who had a live birth and a diagnosis of abruptio placentae were added as a second case group. Subjects are interviewed about six months after their delivery regarding consumption and use of water, type of residential water and other exposures possibly related to stillbirths or abruptio placentae. A residential water sample is collected and analyzed for trihalomethanes and haloacetic acids.

Quality assurance and validation tests were performed on the trihalomethane and haloacetic acids measurement methods using inter-laboratory testing. The effect of travel time, preservative and outdoor air temperature on by-product levels was assessed and data collection methods were modified accordingly.

This is one of the first epidemiologic studies to analyze levels of haloacetic acids from residential drinking water in relation to birth outcomes. The results of this study will provide information about the relationship between exposure during pregnancy to two major groups of chlorination by-products and the occurrence of stillbirths and abruptio placentae. The results may also provide information regarding acceptable limits for levels of trihalomethanes and haloacetic acids.

# Risk assessment of complex mixtures of petroleum-derived PAH on recruitment of fish

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The goal of this project is to enhance the ecological risk assessment of complex mixtures of polyaromatic hydrocarbons (PAH) in oil, creosote, coal tar, and contaminated sediments by developing field and lab methods to assess exposure and cumulative effects, based on chronic toxicity to early life stages of fish that contributes to impaired recruitment.

To assess exposure to PAH, sediment bioassays have been developed that measure the activity of liver mixed function oxygenase (MFO) enzymes in fish exposed for 96 h. MFO enzymes increase in activity after exposure to PAH and metabolize PAH to forms that may be more easily excreted, but sometimes more toxic, than the parent compounds. We have also used chemical analysis of PAH and their metabolites in sediments, bile, and fish tissues to characterize exposure. Lab bioassays for exposure have tested sediments sampled in the field. Because the sediments have been disturbed and mixed, these tests represent "worst case" scenarios of the release of PAH from sediments due to dredging or other disturbances. Field tests involve 96 h exposures of fish in cages suspended in the water column or in contact with contaminated sediments. These tests integrate all factors affecting exposure, particularly the influence of the accumulation of clean sediment caps over contaminated sites. These tests measure the consequences of "leave in place" options for managing contaminated sites.

Toxicity methods have focussed on embryo larval toxicity, on the assumption that those life stages will be the most highly exposed and most sensitive for fish species that are sediment spawners. There have been two basic approaches developed for lab tests, one in which sediments are layered on the bottom of an aquarium with eggs sitting on top, and the second where oil is mixed with a clean reference sediment, to model the effects of spilled oil. In these tests, rates of survival, pathologies (deformities, haemorrhaging, edema), hatch, and growth are compared among exposed and unexposed fish exposed from the day of fertilization to the day when feeding first starts; the duration depends on the species tested. Field tests of toxicity are also being developed, in which fertilized fish eggs are held in cages suspended in the water column, or resting on, or inserted in, sediments. The cages are left in place until hatch and the onset of feeding, and then removed to score the frequency of effects, as indicated above. Exposure of these larvae is characterized by analysis of MFO activity and of PAH concentrations in the test fish, and in sediments from the same site.

Freshwater test species include fathead minnows, white sucker, rainbow trout, and Japanese medaka; marine species were mummichog and 3-spined stickleback. Methods were developed using sediments from naturally and industrially-contaminated sites (Hamilton Harbour, Cornwall, and Barrie, ON; Sydney Harbour, NS, bitumen from tar sands deposits, AL) and from 2-year field studies of the bioremediation of spilled oil (St. Lawrence R., Ste. Croix, Quebec; Petpeswick Bay, NS). Preliminary tests with sexual maturing medaka have also indicated that tests should be developed for endocrine and developmental effects (inter-sex condition, fin erosion).

Overall, this research has generated practical test methods for rapidly assessing the risks to fish of exposure and toxicity of complex mixtures of PAH in sediments. These methods will prove useful in programs of environmental management.

# Effects of maternally-transferred organochlorine contaminants on reproductive success of walleye

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This study applied recently developed techniques to study the effects of organochlorines on the reproductive performance of female Walleye in a contaminated fish population. The effects of these contaminants were also be examined in association with other natural factors that may influence egg quality and/or contaminant toxicity (e.g. lipid content, thiamine content). The research will develop the data necessary to determine the ecosystem health risks associated with known priority POPs, through examining the effects of some of the most toxic POPs, in the natural ecosystem.

# Determination of chloroanilines in milk by solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS)

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Chloroanilines are toxic aromatic compounds and potential environmental contaminants. Possible sources of chloroanilines in the environment include industrial discharges; the metabolism or biodegradation of certain pesticides; and the chlorination of aniline precursors. We have developed an analytical method based on poly(acrylate) fiber solid phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS) for the quantitative analysis of chloroanilines. The analytes examined were 2-chloroaniline, 4-chloroaniline, 2,4-dichloroaniline, 2,6-dichloroaniline, 3,5-dichloroaniline, 2,4,5-trichloroaniline, 3,4,5-trichloroaniline, and 2,3,5,6-tetrachloroaniline. The method allows the sensitive determination of chloroanilines in complex matrices and biofluids. Human milk was studied as a representative biofluid of toxicological importance. A survey of human milk samples did not reveal contamination by chloroanilines.

# Evaluation of the pharmacokinetics and cumulative health effects of disinfection by-product mixtures

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A number of chemicals are formed as a result of the disinfection of water supplies with chlorine or ozone. The by-products of the disinfection process include chloroform, bromodichloromethane, dibromochloromethane, bromoform, trichloroacetic acid, dichloroacetic acid and monochloroacetic acid. When people are exposed to these chemicals in drinking water, the risk assessment is performed by considering the concentration of each chemical individually. In other words, the assessment of health risks associated with human exposure to mixtures of disinfection by-products in drinking water is performed without the consideration of the possible interactions among these substances. Given the properties and characteristics of these chemicals, interactions during absorption, distribution, metabolism and excretion (i.e. pharmacokinetics) may occur. The potential pharmacokinetic interactions among the disinfection by-products have not been investigated. When they do occur, cumulative risk associated with human exposure to disinfection by-products should be assessed in an innovative way that accounts for not only for the effects of individual chemicals but also for their interactive effects. The objectives of this project are: (i) to evaluate the pharmacokinetic interactions among the disinfection by-products occurring as mixtures, and (ii) to evaluate the cumulative adverse effects of disinfection by-product mixtures, accounting for interactions among them. The methodology has involved the conduct of a series of experimental studies to investigate the occurrence and magnitude of interactions among chloroform, bromodichloromethane, dibromochloromethane, bromoform, trichloroacetic acid, dichloroacetic acid, and monochloroacetic acid, and the incorporation of these results within physiologically-based pharmacokinetic models. The results obtained to date indicate that chloroform, bromoform, bromodichloromethane and dibromochloromethane interact with each other, resulting in a significant increase in the blood concentrations of these chemicals in comparison to single administrations. Trichloroacetic acid and dichloroacetic acid have been found to inhibit the metabolism of chloroform, dichlorobromomethane and dibromochloromethane enhancing their blood concentrations in treated animals. These results have been integrated within physiologically-based pharmacokinetic models to simulate the change in the internal doses of the various chemicals during low level mixed exposures. The physiologically-based pharmacokinetic models developed in this study described the animals and humans as a set of tissue compartments interconnected by blood flows. The model simulations of change in internal dose have been used as the basis for conducting cumulative risk assessment for disinfection by-product mixtures. The results of this assessment will be validated by comparing the observations in rats chronically exposed to disinfection by-product mixtures with human epidemiological data. The interaction studies and the modelling methods along with the validation efforts of this project are extremely important in generating new knowledge and major scientific advances to facilitate the understanding and predictability of pharmacokinetics of environmental chemicals in mixtures, such as the disinfection by-products in drinking water. The risk assessment method developed in this study will uniquely account for interactions and potentially resolve the differences between toxicological and epidemiological studies for the effective formulation of health protection policies for disinfection by-products based on sound science.

# Effects of fish consumption from St-Lawrence River on hormonal balance and calcium transport in pregnancy

Julie LAFOND and Donna MERGLER

There is growing concern that low dose exposure to persistent environmental pollutants, such as metals and organo-chlorides (polycyclic biphenyls (PCBs) and pesticides) that enter the food chain, may have subtle but detrimental effects on human health and children's development through their ability to modify endocrine functions. Because of the rapid changes during pregnancy and high level of activity in the placenta, hormonal changes during pregnancy and placental transfer of calcium, can provide insight into the effects of environmental toxins that are normally consumed in our daily diet and lay the basis for our understanding of how these toxins affect children's future development. There is an extensive literature based on animal studies indicating that persistent toxic substances (PTSs) can affect hormonal secretion and offspring development, but only a few studies have addressed this question in human populations. Although these studies have provided important information on the link between in utero exposure and children's neurodevolopment and/or hormonal status, most have focused on separate agents and not on the possible additive or synergistic effects of these substances, which often have similar target organs. The purposes of the present research is to examine exposure to persistent environmental pollutants through food intake and its effect on hormonal secretion and placental transfer in an semi-rural population of pregnant women. We are currently studying a group of pregnant women. By the end of the study, we will have gathered data on 200 pregnant women. These women, have been, and are still, recruited in early pregnancy through the Center for Local Community Services and the obstetrical department of the local hospital. We are examining hormonal status (2nd trimester and at delivery), placental calcium transport with respect to fish consumption and blood concentrations of metals (total mercury, inorganic and organic mercury and lead (Pb)) and plasma organic pollutants (Arochlor 1260, PCB congeners, organo-chloride pesticides, such as DDT, 1,1' (2,2dichloroethenylidene) bis[4chlorobenzene] (DDE) and mirex). Our preliminary results, based on 115 pregnant women, suggest the following: 1) Fish from the St Lawrence River are not the only source of persistent toxic exposure (PTS), but fish from other sources (fresh, canned and frozen) also contribute to higher PTS levels, 2) Several organic pollutants increase significantly between the 1st and 2$^{nd}$ trimester; this was not the case for metals, 3) Organic pollutants increase approximately 5-fold with mother's age (between 15 and 37 years), 4) In the 2$^{nd}$ trimester, prolactine concentration is approximately 50% higher among those with higher levels of organic mercury (2 µg/l); the difference increases with higher levels of Arochlor 1260 and 5) Placental calcium transfer is decreased approximately 40% in women with serum higher levels (>1.0 µg/l) of Arochlor 1260.

These findings suggest that there are several dietary sources of persistent toxic substances and that PTS may influence hormonal secretions during pregnancy and placental transfer of calcium. Further, the different pollutants may have additive or synergistic effects. The findings of this study will allow us to better understand the relation between low level environmental exposure to ubiquitous toxic chemicals and hormonal changes and placental transfer during pregnancy. This information is important in order to act preventively since subtle changes in hormonal balance and calcium transfer can have adverse effects on fetal and childhood development. These results will help regulatory agencies fix standards for food.

# Dissolved oxygen-nutrient interactions and the fate of toxics in microbially based aquatic ecosystems

J.R. Lawrence, J.J. Germida, J.V. Headley, M. Chenier, R. Roy and C.W. Greer

The Toxic Substances Research Initiative (TSRI) has focused concerns on the impacts of various pollutants on ecosystem health. Within this context it is necessary to determine the combined and interactive effects of the many stresses that aquatic habitats receive as a consequence of additions from various sources. Examples of these stresses are nutrients from pulp mills and sewage, metals from mining, hydrocarbons from petroleum development (oil sands) and other sources. Some of the effects of these nutrient sources and contaminants include changes in the levels of dissolved oxygen which may further affect the environment by acting additively, or by acting to mask the effects of each other. Research of this type is necessary to develop meaningful standards and regulations for toxic substances released into the environment and to support or revise our current knowledge and regulations.

To study these questions it is useful to look at organisms that are abundant, everywhere, stationary, that are exposed to stress and critical to the functioning of the ecosystem. Bacteria are perhaps the most diverse group of organisms on the planet accounting for more than 50% of living material, and performing key tasks in ecosystems such as decomposing and recycling all manner of carbon, nutrients and other matter. Bacteria are also responsible for breaking down toxic compounds. Microorganisms, particularly those growing as biofilms on surfaces, (biofilms = bacteria, algae and slimes) are everywhere in aquatic ecosystems and remain at specific locations. As such they represent excellent indicators of changes in ecosystem diversity, health and function. Further, biofilms are a food supply for grazers such as insects and snails, providing an important link in ecosystem food webs.

We used confocal laser microscopy and image analyses combined with molecular biology techniques to collect information on how nutrients, selected toxic compounds and oxygen level influenced the growth of bacterial biofilms from rivers. Experiments were carried out using model systems to simulate conditions in rivers and carefully control the treatments. The studies indicated that the approaches used provided important information about the fate of toxic compounds in rivers. We found that the presence of nutrients significantly influenced the fate of metals and organic contaminants such as pesticides and petroleum products. For example, the presence of nutrients increased the sorption of contaminants to biofilms. These studies confirm the importance of studying interactions between nutrients and contaminants. It was also found that the responses of the microbial community depended on the season (summer or winter) in which they were developed . In this case summer biofilms degraded less than 5% of a petroleum contaminant whereas winter biofilms degraded up to 70% of the same contaminant. Some microorganisms that specifically accumulate, metals such as nickel, or toxic organic contaminants were identified in river biofilms. The presence of these accumulator organisms is very important for the transfer of contaminants to the insects and other aquatic life that feed on microbial biofilms. Thus the presence of nutrients and the composition of the bacterial community have a significant impact on the fate of toxics in river biofilms, and this can be used as an indicator of the impact of human activities on natural ecosystems.

# Environmental effects and remediation of contaminants in Sydney Harbour, Nova Scotia

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Four oceanographic research expeditions have been conducted under this project to collect samples of sediments, water and biota to determine concentrations and the partitioning of contaminants (dissolved and particulate metals - Cd, Cu, Fe, Ni, Pb, Zn, Mn, Hg), and organic contaminants - PAHs and PCBs) within Sydney Harbour, Nova Scotia - one of the most hazardous toxic waste sites in Canada. While the concentrations of dissolved metals were not found to exceed marine water quality guidelines for protection of aquatic life, elevated values for sediments were frequently reported in the South Arm. The highest concentrations were observed around the mouth of Muggah Creek, a major point source for polycyclic aromatic hydrocarbons (PAHs) into the harbour.

The historical record of contaminant inputs to Sydney Harbour has been determined by chemical analysis of dated sediment cores. Both PAH and PCB concentrations had maximum values in the 1960s and 1970s. Recent sediment deposits are less contaminated. These results and that from concurrent physical and geological studies under this project will be used in models to identify the impact of natural processes on the retention of residual contaminants within Sydney Harbour.

An objective of the current program is to quantify the ecological risk and bioavailability of contaminants in sediments from Sydney Harbour. In addition to quantitative analysis of the seabed biological organisms >0.5 mm (macrofauna) in sediment samples, sediments from Sydney Harbour were evaluated with a battery of biotests. These results will be used to identify the primary factors (both natural and as the result of human influence) that affect benthic community structure. In terms of natural recovery, studies have also confirmed that natural microbial processes influence the biodegradation and biotransformation rates of the contaminants of concern. Linkages among the multiple assessment indicators (chemical analysis, biological effect data and benthic community structure analysis) are being used in a weight of evidence approach to grade environmental conditions within Sydney Harbour and its approaches. Data from this project will be compared with available historical data for trend analysis. Furthermore, data has also been transferred to other agencies such as Health Canada that are conducting an assessment of human health impacts associated with regional industrial contaminants.

Studies in physical and geological oceanography have been completed within Sydney Harbour. This data has improved our understanding of the role that circulation plays in the distribution of bottom and water-borne contaminants. These results have been incorporated into a mass balance model formulated with the chemical and biological data from this program as well as archived data. The model outputs will provide scientific data needed by environmental resource managers who must weight the advantages of proposed remedial activities within the region against natural attenuation (no treatment).

# Distribution and effects of pharmaceutical drugs in the Canadian aquatic environment

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In Europe, a variety of prescription and non-prescription drugs have been identified in the effluents of sewage treatment plants (STPs) and in rivers and streams near STP discharges. Chronic exposure of aquatic organisms to drugs discharged from STPs could alter their physiology, behaviour or reproductive capability. The primary objectives of this one-year project were to determine whether prescription and non-prescription drugs occur in the Canadian aquatic environment and to determine whether drugs can induce biological effects in fish. In a survey of the effluents from STPs in 14 Canadian cities, analgesic/anti-inflammatory prescription drugs such as Ibuprofen and breakdown products of aspirin (salicylic acid), as well as several prescription drugs were detected in STP effluents at part per billion concentrations. In general, the concentrations of drugs in STP effluents were greater than those observed in Europe; probably because sewage treatment is often not as extensive in Canada as it is in Europe. Some drugs, such as the anti-epileptic, Carbamazepine appear to be very resistant to degradation in STPs. Samples of surface water were collected in the summer and fall of 2000 at open water sites in Lake Ontario and Lake Erie, and at sites near the STPs of the cities of Windsor and Burlington/Hamilton. At sites near the Little River STP in the city of Windsor, concentrations of drugs were high at locations as much as 600 meters downstream of the STP. Since the effluent from this STP contributes approximately 50% to the total flow of Little River, high concentrations of drugs in this river were expected. In samples of surface water collected near the STPs in Hamilton Harbour and near the West Windsor STP, which discharges directly into the Detroit River, concentrations of drugs declined rapidly within a hundred meters of the STP discharges. These data indicate that drugs are detectable only close to the point of discharge from STPs, unless the effluents are discharged into small rivers. Various molecular and biochemical tools were developed to study the biological effects of Gemfibrozil and Carbamazepine on goldfish. This model fish species was used because the necessary biochemical and molecular research tools were available for this species. When goldfish were exposed to the antiepileptic, Carbamazepine, levels of growth hormone were increased in the blood. In goldfish exposed to the cholesterol-regulating drug, Gemfibrozil, cells of the liver and kidney showed evidence of damage and chemical secretions in the brain were altered. These data demonstrate that drugs can alter the balance of hormones and affect cell function in fish. These molecular and biochemical markers will now be applied to monitoring wild fish in lakes and rivers impacted by STPs. Overall, this study has shown that the environmental contamination by prescription and non-prescription drugs occurs in Canada and this contamination has the potential to affect wild populations of fish in areas near sewage treatment plants.

# URB-MAN: A Habitat-Based Risk Assessment Tool for Managing Toxic Chemical Exposure in Urban Fisheries

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This research contributes to the development of a risk assessment tool to reduce human exposure to toxic chemicals while enhancing the productivity of the fisheries. The research will predict the effects of anthropogenic activities namely, physical changes to aquatic habitat, on the exposure of fish and consumers of fish to toxic chemicals. URB-MAN represents a unique approach to risk assessment because it simultaneously considers the effects of changes in physical habitat structure on fish community composition, abundance and exposure to contaminants by fish and consumers of fish.

# Interactive prenatal toxicity and neurobehavioral effects of concomitant gestational exposure to methylmercury and polychlorinated biphenyls

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Inuit people are highly exposed to methylmercury (MeHg) and complex mixtures of organochlorines including polychlorinated biphenyls (PCBs), *via* seafood consumption. Each of these neurotoxic agents was shown to alter fetal and neonatal brain development when tested alone in several animal studies. Little is known, however, on possible interactions between these neurotoxicants when administered concomitantly. Until recently, epidemiological data suggested independent effects of developmental exposure to MeHg and PCBs on several neurobehavioral endpoints, apparently affecting different functional domains (reviewed in Rice, *Neurotox.* **21**, 1039-1044, 2000). It should be kept in mind, however, that interactions between dietary toxicants often cannot be detected in such studies due to intercorrelations between compounds, which make it difficult to separate the effect of each agent. A recent study of neurobehavioral deficits associated with PCBs in 7-year-old children prenatally exposed to seafood neurotoxicants in the Faroe Islands now suggests that the PCB-associated neurobehavioral deficits detected within the highest tertile of mercury exposure involved a possible interaction between methylmercury and PCBs (Grandjean et al., *Neurotox. Teratol.* **23**, 305-317, 2001).

Here, the mouse was used as an experimental model to assess the effects of gestational exposure to an *Arctic-relevant* PCB mixture, MeHg or a combination of both on perinatal development and offspring neurobehavior. Concomitant administration of subtoxic doses of PCBs and MeHg in the third quarter of gestation dramatically increased perinatal mortality, as compared to treatments with each toxin alone. The survival data show that PCBs and MeHg interacted synergistically to increase mortality at doses which did not affect body weight gain from birth to weaning in survivors. In contrast, behavioral testing of surviving offspring failed to reveal synergism following concomitant exposure to both neurotoxicants. Although the distance traveled, frequency of rearings and time spent in the center of an open field, as well as reference memory in a Poucet T-maze delayed alternation task, were differentially affected by each neurotoxic agent, there was no evidence for interactive effects between PCBs and MeHg on exploration and memory. This study suggests, for the first time, that methylmercury and PCBs interact synergistically on perinatal lethality, but not neurobehavioral deficits in surviving litters. The interaction between MeHg and PCBs in perinatal brain development will have to be further investigated at lower doses, using molecular and morphological endpoints in addition to broader batteries of behavioral tests. These first results raise the possibility that a concomitant exposure of pregnant women to these pollutants may increase the risk of perinatal mortality in highly exposed populations, in addition to causing neurobehavioral deficits in children. This work was supported by a one-year TSRI research grant (#286).

# Development of a cumulative effects assessment strategy for the Saint John River

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We have been developing a strategy for dealing with cumulative effects assessment, which uses the performance of resident fish to help understand the level of stress on a river reach. This study is examining the performance of slimy sculpin, white sucker and yellow perch in the upper Saint John River basin to develop a strategy for increasing the level of understanding of the freshwater portion of the river system. The data will be used to give a relative indication of performance within the system and to prioritize areas of concern within the river basin.

The first year of the project (1999) collected data from more than 30 sites in the upper river basin. Slimy sculpin were effective at discriminating impacts of closely related discharges on the Saint John River. Agricultural practices were associated with an increased growth rate of sculpin, and a decrease in the abundance of young-of-the-year. The second year of the project (2000) documented fish community structure throughout the freshwater portion of the basin, and expanded the information base on tributary drainages. Third year TSRI studies included an examination of fish performance in 25 tributaries in the upper basin, as well as initiating detailed studies in the middle reaches of the river.

The final analyses will evaluate the relative strengths of fish and benthic community data, population estimates and fish performance evaluations, for determining the health of the system. Surveys will be conducted on resident fish, habitat suitability and fish and invertebrate communities, to develop a baseline of performance for interpreting other studies. There are more difficulties in dealing with estuarine and marine areas, and preliminary work will enable eventual expansion of the framework to downstream areas.

The relative differences between fish performance in reaches of the river were compared to document which sections of the river were priorities for follow-up studies. For example, while growth rates of fish downstream of the pulp mill at Edmundston are 50% higher than upstream reference sites, the size of young-of-the-year fish in potato-farming areas is 600% larger than upstream reference sites. Original discussions at the initiation of the studies suggested that priority stressors would be pulp mills and hydroelectric facilities. Performance comparisons have shown that there are priority concerns associated with poultry processing facilities in the upper basin, potato-farming areas in tributaries and potentially with small effluent discharges in the middle reaches of the river. These sites were not suggested as priorities based on initial discussions.

Cumulative effects assessment can be retrospective (describing existing condition) or predictive. Existing approaches to multiple stressor assessment are predominantly stressor-based, depending on existing knowledge of biological interactions and stressor effects. Our studies are showing that there are a variety of concerns and impacts that have been ignored by focusing on traditional approaches. We are developing an effects-based approach that offers the potential to provide a common framework for environmental impact assessment, cumulative effects assessment, environmental effects monitoring, and surveillance monitoring.

The project has received funding from more than 20 additional small grants (< 25K) that would have not been sufficient on their own to conduct significant field work. In addition, in kind support was received from more than 25 partners within the basin. The partnership funding has totaled more than 800K in cash and 3.4M$ in in-kind support.

# Exposure and endocrine impact of organochlorines in a Native community

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The Mid-Canada Line was one of several extensive surveillance networks operating in Canada during the Cold War and consisted of 98 radar sites spread across the 55th parallel. The sites were closed in 1965 and many were abandoned leaving behind electrical equipment containing PCB-laden fluids and soils contaminated with organochlorine pesticides (especially DDT). An example of this was the station on Anderson Island, adjacent to the Fort Albany First Nation Cree Community. With this as a background, our research project had two objectives. First, we set out to determine the exposure to PCBs and DDT of the inhabitants of Fort Albany by measuring the concentrations of these contaminants in blood (plasma) samples. For this purpose we used two control groups, one at the neighbouring First Nations Cree community of Kashechewan and the other in Hamilton, Ontario, Canada. In the two Native communities, 50 each of adult males and females donated blood samples, and 25 of each in Hamilton, ON. It is clear that for both males and females the levels increased in the order : Hamilton, Kashechewan, Fort Albany. Interestingly, the Fort Albany DDT results corresponded to the higher end of the range of geometric means observed among high-Arctic Inuit peoples. By comparison, the Fort Albany PCBs values matched the lower end of the concentration range observed for the mentioned Inuit groups. Clearly, the data suggest a point source of these contaminants in the Fort Albany community. It should be stated that our sample collection preceded the cleanup of the soils on Anderson Island.

The second component of our research project constituted a pilot study of the possible impact of organochlorines on menstrual cycle endocrine endpoints. Nine volunteers donated 42 consecutive daily urine samples and kept a diary about menstrual cycle details. The urine samples were analysed for hormones (or their metabolites) involved in regulating the menstrual cycle (e.g. estrogen, progesterone, follicle stimulating hormone, FSH, and luteinizing hormone, LH). As before, exposure to PCBs and DDT were assessed by their blood plasma levels. Many menstrual cycle endocrine endpoints involving the mentioned hormones were constructed for the follicular and luteal phases of the menstrual cycle, such as the timing of concentration surges in relation to ovulation. Such endocrine endpoints were then correlated with the plasma concentrations of the indicated organochlorines. A number of statistically significant associations were observed, as well as quite a number of near significant relationships. The nature of the associations suggest a repression of circulating estrogen which enhances concentrations of FSH and LH. It is obvious that these findings need to be confirmed before cause-and-effect interpretations are warranted (i.e., increased sample number, adjustment for age, breast feeding, etc.). Nevertheless, menstrual cycle endocrine endpoints show promise as non-invasive and sensitive biomarkers of the impact of low-level organochlorines exposures on fertility.

# Determining the effects of environmental contaminants on mutations of repeated DNA sequences in mice

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Animal genomes contain many repetitive regions of DNA which undergo a high rate of mutation during passage from one generation to the next. This high variability greatly improves probability of observing induced mutations when investigating effects of environmental contaminants on germinal cells (eggs & sperm). Previous studies (Yauk, Quinn) of Great Lakes herring gulls demonstrated a significant increase in the rate at which mutant repeat patterns arose in the offspring of populations exposed to high levels of industrial pollution near steel mills. Mutations of this kind have been associated with human diseases, including epilepsy, diabetes, and cancer. One direction of the present study was to determine the effect of existing levels of environmental contaminants on the rate of inherited repeat DNA mutations in laboratory mice. These animals are very often used as models for human health effects. Male and female mice in cages were exposed to the local air for 10 weeks at a location immediately adjacent to an industrial area containing 2 steel mills, and later mated. Data from the first experiment indicate a statistically significant doubling of the frequency of mutant repeated DNA sequences in the offspring of the mice exposed to air near the mill, compared to offspring of mice held in a clean air location. Analysis of data from a second experiment is pending.

Repeat DNA sequence variation also arises during cell divisions in non-germinal cells of the body, where the influences of chemical agents are more easily studied than in the longer animal mating experiments. The second part of this study employs laboratory-grown tissue cells, to determine if any environmental contaminants can preferentially generate mutations in repeat DNA sequences. Conventional laboratory tests for genetic hazards most often used for Government regulatory purposes do not specifically monitor mutations in repeated regions of DNA. This omission could result in overlooked health risks, such as cancer, for some chemical agents. Our comparison of induced mutations in repeated DNA sequences with responses in conventional genetic toxicology assays was begun by generating and characterizing two epithelial cell lines (one from mouse lung, one from rat mouth) that have an easily detectable target gene of non-repetitive DNA constructed in their genomes. Results to date show that benzo[a]pyrene, a chemical often found in steel mill emissions, damages chromosomes and is mutagenic at both repeated and non-repeated DNA sequences. These results contrast with those of mitomycin C, which damages chromosomes, is mutagenic to the non-repetitive target gene, but is not mutagenic in the repetitive DNA sequence chosen for study. Additional studies of chemicals that are unlikely to directly damage DNA or chromosomes are underway. We expect to find chemical agents that have little effect on regions of non-repetitive DNA or chromosomal structures, but can be shown to be effective at inducing changes in repeated DNA regions. Tests for mutations in repeated DNA may become necessary considerations in future regulatory assessments of environmental agents.

# Toxicological risk associated with the use of ethanol in automotive gasoline

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Much of the greenhouse gas that is contributing to the warming of the planet is produced by the transportation sector through gasoline combustion in motor vehicles. Many approaches to reducing such emissions have been proposed, including the addition to gasoline of a certain amount of ethanol, the form of alcohol found in alcoholic beverages. In fact, it has been demonstrated that adding oxygenated products such as ethanol, improves fuel combustion while reducing emissions of various pollutants, including carbon monoxide and formaldehyde. However, the addition of ethanol is known to lead to the increased production of certain pollutants, including acetaldehyde, an irritating and highly toxic substance. Acetaldehyde is also produced in the body by enzyme transformation of ethanol, and is in turn broken down into harmless products. In this way, when an individual consumes an alcoholic beverage or inhales ethanol vapours, a certain quantity of acetaldehyde is produced in his or her body. Although ethanol toxicity, like that of acetaldehyde is recognized in humans, little is known about the consequences of inhaling ethanol or acetaldehyde vapours on resulting blood levels, or about the effects of such exposure on human health.

It is anticipated that the use of gasoline containing varying levels of ethanol will result in increased exposure of the public to ethanol and acetaldehyde. The purpose of this project is to lay the foundations for a research program aimed primarily at providing Health Canada managers with tools for predicting biological levels of ethanol and acetaldehyde and the toxic hazard to the general public, and particularly to individuals who could be especially sensitive, of exposure to ethanol and acetaldehyde fumes. Certain individuals have a genetic deficiency involving the aldehyde dehydrogenase enzyme and the elimination system. This deficiency reduces their capacity to break down acetaldehyde produced from ethanol, thereby exposing them to high levels of acetaldehyde and to the toxic effects of this substance.

Through our laboratory work, we have 1) described the behaviour of ethanol in an animal model, the laboratory rat, exposed to various inhalation concentrations of ethanol, 2) demonstrated that exposure to ethanol can affect lung tissues, and 3) developed a technique for studying the impact of the enzyme deficiency described above on the toxic hazard to sensitive populations of inhalation exposure to ethanol and acetaldehyde. Future research should enable us to improve our understanding of the health risks that may result from increased use of ethanol in gasoline.

# The relationships between traditional Innu harvesting practices, environmental contaminants and wildlife and human health/ exposure

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This study examined for a relationship between gross indicators of individual health of animals harvested by Innu in southern Labrador, and the levels of contaminants in these animals and the determination of human exposure levels due to consumption of contaminated country foods by the Innu people.

# Effects of polonium-210 alpha radiation on human and animal systems

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Exposure to alpha radiation from ingested polonium-210 (Po-210) is one of the most significant sources of radiation exposure in caribou and in northern human populations who are dependent on caribou for a major portion of their meat supply. Po-210 is a naturally-occurring radionuclide, resulting from the decay of radon gas in air. It gradually settles onto slowly-growing lichens which serve as the main food source of caribou during the long Arctic winters. We now have good information on the concentrations of Po-210 in caribou meat and organs, in the amounts consumed by northern residents, and in the fate of the polonium once it enters the human body. What we don't know is whether current levels of Po-210 are causing any health effects or biological changes in humans and animals from northern Canada.

This project is a multi-disciplinary effort. Scientists from the University of Saskatchewan have irradiated cultured aorta cells from cows with x-rays and with alpha particles from Po-210. These cells were chosen because they are easy to collect, easy to grow in a culture medium, and are a good surrogate for effects on caribou in the wild. We found that alpha radiation from Po-210 is ten times as effective as x-rays in killing the aorta cells and in preventing them from reproducing. The cells which line the aorta and other blood vessels are not particularly susceptible to cancer induction. However, there is recent evidence from A-bomb survivors and Chernobyl emergency workers that radiation can cause cardiovascular disease. Our results will help to establish this link.

Experiments on lymphocytes (a type of white blood cell) from cows are now nearly complete. These radiation sensitive cells, if damaged, may compromise the immune system responses. The next step is to collect blood samples from caribou and test their lymphocytes for radiation sensitivity.

Scientists at Health Canada are using the latest techniques to measure chromosome aberrations and abnormalities in human blood cells that may be caused by radiation. In the early winter we will be collecting blood samples from a northern community where a large amount of caribou meat is consumed. The cells will first be tested for possible damage from current environmental exposures to radiation. Then the cells will be bombarded with artificial radiation to determine whether they are more sensitive or less sensitive to radiation damage as compared to blood cells taken from people who do not eat caribou meat. The results will help decide whether more stringent standards of radiation protection are needed for northern residents. The results will also help to show if northerners are being impacted by exposure to a mixture of toxic substances in their environment — whether these substances arise from local mining operations or from long range atmospheric transport of industrial pollutants from the south.

# Immune function and stress response in relation to trace element exposure in wild, arctic sea ducks

Wayland, Mark and Smits, Judit

There is growing evidence that populations of sea ducks are declining in the Arctic. It has been suggested that contaminants, especially metals, may be one of the environmental stressors contributing to these declines.

In some communities in the Canadian Arctic, sea ducks are an important traditional food. Declining populations could impact the harvest of these birds. In addition, high contaminant levels could detract from the perceived quality of sea ducks as traditional foods.

Because of our concerns about sublethal effects of contaminants on sea ducks, we investigated relationships between metal concentrations and certain measures of health and fitness in a representative species of sea duck, the common eider. Specifically, we examined relationships between tissue mercury, cadmium and selenium concentrations and the integrity of the immune system, the hormonal response to stress, vitamin A levels in plasma and liver and overall body condition.

The study was done at the East Bay Migratory Bird Sanctuary on Southampton Island, Nunavut.

Overall, we found no consistent evidence that tissue metal concentrations in these birds were adversely affecting their health and fitness. It is unlikely that metals are contributing to population declines by weakening the resistance of these birds to other types of environmental stress. Nevertheless, further investigations are needed to determine whether the inverse relationship between body condition and mercury concentrations that we observed, was the result of a cause-and-effect relationship.

A human health assessment concerning the levels of trace metals in livers and kidneys of sea ducks in the arctic was recently completed by Health Canada. The results of that assessment were sent to the Nunavut and NWT Contaminants Committees for dissemination to the communities.