*Water Qual. Res. J. Canada, 2001*
*Volume 36, No. 2, 215–231*


# A Review of the Evidence for Endocrine Disruption in Canadian Aquatic Ecosystems

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**Endocrine disrupting substances in the environment and the potential affects they have on wildlife species has recently received increased public attention. This paper provides background information on research that has addressed the endocrine disruption issue in the Canadian aquatic environment as well as information on studies that are presently being conducted within the country to address this issue. Two of the three studies from across the world often cited as presenting sufficient evidence for connecting contaminants and endocrine disruption in fish populations are Canadian — Lake Ontario lake trout and TCDD and related compounds, and white sucker exposed to bleached kraft pulp mill effluent. Several other Canadian examples exist, including altered stress responses in yellow perch exposed to heavy metals, altered smoltification in Atlantic salmon exposed to 4-nonylphenol and imposex in dogwelks exposed to tributyltin. While other Canadian studies suggest alterations in reproductive function in fish, direct links to contaminants have not been made. Other studies have identified endocrine active compounds in the receiving environments but have yet to link these to alterations in endocrine function in resident fish populations. The strength of Canada's research programs lies in the breadth and depth of their field related research. It is this world-recognized expertise and strength that Canada can contribute to the international effort to address the endocrine disruptor issue.**

*Key words:* **fish populations, growth, reproduction, Great Lakes, national agenda**

## Introduction

The issue of endocrine disrupting substances (EDS) in the environment has received increased attention over the last several years. This is due, in part, to recent publications that provide information on potential links for chemicals in the environment to altered reproductive health and developmental abnormalities in human populations (Colborn et al. 1996). Although direct cause and effect studies are difficult to conduct in humans, a great deal of the existing scientific information that links chemical exposure to altered endocrine function has been provided by studies conducted on fish and wildlife. In order to establish cause and effect, a weight-of-evidence approach is often used. Ankley and Giesy (1998) set their specific criteria to include the demonstration of an effect, establish-

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ment of a plausible mechanism of action, the identification of the chemical(s) involved, a reasonableness of exposure and some evidence of recovery following removal from this exposure.

Over the last four decades, scientists at the Canadian Wildlife Service have been conducting studies on developmental and reproductive alterations in a number of avian species, relating these effects to specific contaminants and following recovery in these functions following declines in contaminant exposure (Fox 2001). Declines of a number of salmonid fish species were also evident in the Laurentian Great Lakes beginning in the early 1940s, although the specific cause(s) of these declines (habitat alterations, lamprey predation, overfishing or contaminants) is still debated. As part of the Canadian federal government's development of a program to address the issue of endocrine disruption, a workshop was held to establish a national agenda for the scientific assessment of endocrine disrupting substances (Servos et al. 2000a). This paper focuses on endocrine-related research conducted within Canadian aquatic ecosystems, particularly on fish studies that provide evidence for alterations in endocrine-controlled functions related to contaminant exposure.

While survival, growth and reproduction in fish populations may have been affected by a number of contaminants in a number of locations within Canada, it is often difficult to determine the role of endocrine disrupting compounds in these effects as, often, a number of other variables or factors may be involved. Environment Canada under its *Canadian Environmental Protection Act, 1999* (CEPA 1999) has defined a hormone disrupting substance as a substance having the ability to disrupt the synthesis, secretion, transport, binding, action or elimination of hormones in an organism or its progeny, that are responsible for the maintenance of homeostasis, reproduction, development or behaviour of an organism (CEPA 1999). Leatherland (1993) provided examples of effects that have occurred in wild fish species and that have been classified as potential endocrine disruption effects. These include thyroid enlargement, decreased thyroid hormone content, greater prevalences of premature sexual maturation in males, loss of secondary sex characteristics, decreased plasma gonadotropin and gonadal hormone content, reduced egg fertility, feminization of males, defeminization of females, intersex, changes in steroid sex hormones, induction of certain biomarkers such as plasma vitellogenin, and increased embryo mortality and deformities. However, by their nature, fish are often more difficult to study than are mammals as it is difficult to conduct controlled studies that are relevant to wild populations. It is also difficult to separate out the effects of contaminants on endocrine endpoints from other environmental, nutritional, and compensatory effects.

## Evidence of Endocrine Disruption in Canadian Aquatic Ecosystems

Over the last several years, numerous meetings have been held and a number of publications have been written on endocrine disruptors and their relation to effects in aquatic life. One of the first meetings that helped establish endocrine disruption as an important environmental issue regarding aquatic ecosystems was the 1995 Wingspread workshop on "Chemically Induced Alterations in Functional Development and Reproduction in Fishes". In the proceedings from the workshop (Rolland et al. 1997), Monosson (1997) discusses the reproductive and developmental effects of contaminants in fish populations and attempts to establish cause and effect with certain case studies. The participants identified these studies as the best known examples of field research on reproductive and developmental effects of contaminants on fish populations with supporting laboratory confirmation of the identified responses. Of the studies identified, two were Canadian. This included blue sac disease and increased larval mortality in lake trout in Lake Ontario caused by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. The second involved decreased sex steroids and gonadal development in Lake Superior white sucker exposed to bleached kraft mill effluent. Another review of endocrine disruptors in wildlife, a weight-of-evidence perspective (Ankely and Giesy 1998), also cited these two previous examples as two of the three studies from across the world that presented sufficient evidence connecting contaminants and endocrine disruption in fish populations. They also state that there is other evidence that the endocrine systems of fishes are impaired in aquatic ecosystems, but at this time it is not clear whether these observed imbalances are the result of exposure to xenobiotics (Giesy and Snyder 1998).

### Great Lakes Salmonids

The Great Lakes fishery has changed dramatically since the early 1900s. Commercial catches of historically important species such as lake trout, whitefish, herring, blue pike and ciscoes have declined and have been replaced by smelt, chub, eels, yellow perch, white bass, bullhead and suckers. In a review of toxic chemicals in the Great Lakes and their associated effects (Government of Canada 1991), it was indicated that stocking programs, sea lamprey control, habitat conservation, quota management and reductions in inputs of phosphorus and persistent toxic chemicals have reversed some of these trends. However, some important species have not recovered, such as the lake trout.

There has been a great deal of investigation into the possible effects of organochlorines on reproductive status of Great Lakes fishes, in particular salmonids. In general, however, it has been difficult to link observed reproductive effects to any particular contaminant or group of contaminants. One exception is an analysis recently completed by scientists from the U.S. Environmental Protection Agency and the University of Wisconsin. They used a progressive series of laboratory and field studies to examine relationships between historical trends in contaminant levels and reproductive success of lake trout in Lake Ontario (Cook et al. 1994, 1997). In the early 1940s, lake trout populations in the Great Lakes declined abruptly and by the mid-1950s were deemed extinct throughout the Great Lakes, except for isolated populations in Lake Superior (Cook et al. 1997). The decline was originally attributed in large part to overfishing, lamprey predation and habitat modification. Although sports fisheries were established though stocking programs, attempts to establish naturally reproducing lake trout populations met with little success due to a lack of recruitment. Further studies using artificially spawned adults collected from Lake Ontario in the 1970s indicated sac fry mortality prior to swim up, now known as blue-sac disease, as the major cause. In-depth laboratory studies determined a relationship between blue-sac disease and aryl hydrocarbon receptor (AhR) agonists such as 2,3,7,8-tetracholordibenzo-p-dioxin (TCDD) and related compounds (Walker et al. 1994). Toxic equivalent factors (TEF) were then determined for these compounds in eggs that would result in blue-sac disease. These TEFs could then be used to evaluate fish eggs for TCDD and related compounds to predict potential for early embryo mortality. However, historical data for these compounds in eggs were not available, so Cook et al. (1997) used measurements of residues of these compounds in dated sediment core samples from Lake Ontario to determine when survival of sac fry would be expected. The predictions made using this approach were in excellent agreement with observed trends in lake trout reproduction in Lake Ontario, including recent signs of successful reproduction, indicating a likelihood that in the past, AhR agonists were primary contributors to adverse population-level impacts in this system (Cook et al. 1997).

Recent studies have provided some insight into the underlying mechanisms by which AhR agonists may induce blue-sac disease (Cantrell et al. 1996). They demonstrated that AhR-induced production of CYP1A resulted in lipid oxidation of cell membranes, leading to programmed cell death (apoptosis), followed by vascular damage and subsequent embryo mortality. Thus, while various signaling pathways may be involved, it is unlikely that blue-sac disease as observed in Great Lakes fish is due to endocrine disruption as it is currently defined (Giesy and Snyder 1998), in terms of disrupting the synthesis, secretion, transport, binding, action or elimination of hormones.

Since their introduction into the Great Lakes in the late 1950s and early 1960s, some Pacific salmon stocks have exhibited a variety of endocrine, reproductive and developmental abnormalities. The one effect that has been the most prominent in the Great Lakes is altered thyroid function, including thyroid enlargements, thyroid hypertrophy and/or hyperplasia, thyroid tumors and goiters. Leatherland (1993) found thyroid hyperplasia in every single adult specimen examined, regardless of the lake of origin, year of collection, or species. The original prognosis as iodide deficiency has since been disproved and it is now thought to be some form of waterborne goitrogen, although studies have eliminated the halogenated aromatic hydrocarbons such as PCBs and dioxins (Leatherland 1993). The exact cause of these alterations in thyroid function and the direct link of xenobiotic effects on thyroid function and hormones have yet to be definitively demonstrated (Giesy and Snyder 1998). Other alterations in salmon stocks include increased frequency of precocious sexual maturation, loss of secondary sexual characteristics in male fish, reduced levels of gonadal steroid hormone secretion and reduced egg fertility. However, interpretation of the links of these effects to reproductive and developmental dysfunction has to be made with caution as the evidence linking these effects to contaminants is not as great as that for thyroid dysfunction (Leatherland 1993).

### Effects of Pulp and Paper Mill Effluent on Endocrine Function and Associated Reproductive Processes

Probably the best example of endocrine disruption in fish within Canada is that of alterations in reproductive function downstream of some pulp and paper effluent discharges. Perhaps the most extensive of these studies are those on white sucker (*Catostomus commersoni*) exposed to bleached kraft mill effluent (BKME) in Jackfish Bay, Lake Superior. Initial studies at this site indicated a number of abnormalities, including reductions in gonadal size, delayed sexual maturation and reduced expression of secondary sexual characteristics (McMaster et al. 1991, 1992; Munkittrick et al. 1991). White sucker of both sexes had reduced levels of the dominant sex steroid hormones throughout the period of gonadal development. More in depth reproductive assessments were conducted during their spawning migration and identified a number of sites within the reproductive-endocrine axis affected by exposure to BKME (Van Der Kraak et al. 1992). Impacts included altered pituitary function (reduced circulating levels of gonadotropin-II (GTH-II) and diminished GTH-II response to a superactive gonadotropin-releasing hormone analog), reduced ovarian steroid biosynthetic capacity and altered peripheral steroid metabolism. Despite these reproductive responses, exposure to BKME did not affect fertility of the eggs or sperm obtained from fish reaching sexual maturity nor subsequent larval development (McMaster et al. 1992). Continued studies at this site identified alterations of in vitro steroid productive capacity as one of the dominant alterations in reproductive function. These alterations in steroid production were due to reductions in steroid substrate availability and altered steroid biosynthetic enzyme efficiencies (McMaster et al. 1996a). Additional detailed mechanistic studies identified increases in gonadal apoptosis and altered stress protein (hsp70) expression (Janz et al. 1997) and increased levels of gonadal oxidative stress (Oakes et al. 1999), all possibly contributing to reductions in gonadal steroid productivity. Further studies identified alterations in sex steroid binding protein levels, increased hepatic oxidative stress (Oakes et al. 1999), reductions in female circulating vitellogenin levels (corresponding to reduced 17β-estradiol levels), altered hepatic estrogen and gonadal androgen receptors, and differential liver steroid metabolism (Squires et al. 1998), but similar production levels of gonadal prostaglandins (Van Der Kraak et al. 1992). All of these studies clearly support the conclusion that pulp mill effluent from this site is responsible for the reproductive endocrine alterations. Other supporting evidence was collected following a short-term shutdown at the mill, where fish showed signs of recovery in terms of circulating sex steroid levels and hepatic EROD activity following no exposure to the effluent for up to 2 weeks (Munkittrick et al. 1992a). Goldfish exposures to effluent from this site also demonstrated a reduced potential to produce steroids in vitro relative to goldfish held in reference site water (McMaster et al. 1996b).

Interestingly, a comparison of fish responses to pulp mill effluent, 17β-estradiol, and the plant sterol, β-sitosterol, identified some similar mechanisms of alteration on reproductive function. However, male fish collected downstream of pulp mill effluent did not demonstrate induction of plasma vitellogenin, a classic estrogenic response, that was induced by the plant sterol and 17β-estradiol, suggesting that effluent impacts were not necessarily due to estrogenic properties (Van Der Kraak et al. 1998). Currently, there is an effort underway to use toxicity-based fractionation approaches, similar to those utilized by Brighty and coworkers for sewage effluents (Brighty 1997), to define the specific chemicals causing these reproductive effects in fish exposed to pulp mill effluent at the Jackfish Bay site (Hewitt et al. 2000).

These reproductive effects (reduced gonadal development, decreased steroid levels) have also been demonstrated in other species at this site (Munkittrick et al. 1992a,b) as well as at other sites within Canada (Munkittrick et al. 1994; Hodson et al. 1992, McMaster et al. 1996a, Servos et al. 1992; Gagnon et al. 1994), although the detailed reproductive evaluations have not been conducted at all of these locations. Following the Northern River Basins Study, which identified evidence of sex steroid depressions in fish and an increased number of sexually immature fish below some of the pulp and paper mills within this basin (Canada-Alberta-Northwest Territories 1997), a detailed endocrine evaluation of wild fish in these areas was initiated. This new study, funded through Canada's Northern Rivers Ecosystem Initiative and the Toxic Substances Research Initiative, will utilize techniques that have been developed over that last number of years of study at Jackfish Bay. This includes the measurement of circulating sex steroid levels, gonadal steroid biosynthesis, circulating vitellogenin levels, ovarian apoptosis, hepatic and gonadal sex steroid receptors, sex steroid binding proteins, gonadal histology (for potential intersex) as well as stage of gonadal development, hepatic mixed function oxygenase activity, circulating and pituitary gonadotropin content as well as the normal measures of reproductive health including gonadosomatic indices and fecundity estimates. With this large array of endocrine endpoints being analyzed simultaneously, the impacts of modern pulp and paper mills in Canada on endocrine-related functions in fish in the receiving environment will be determined.

## Additional Studies Identifying Endocrine Alterations in Canadian Aquatic Life

The following is a compilation of the work being conducted at this time on endocrine disruption in Canadian aquatic ecosystems.

**Municipal Wastes**

In the mid-1980s in the United Kingdom, a research team led by John Sumpter sought to address possible causes underlying the observation of hermaphroditic fish caught by anglers from waters below sewage treatment plants. They hypothesized that one factor contributing to the phenomenon was the presence of estrogenic substances found in effluents (Purdom et al. 1994). Recent work by this group revealed that in many effluents the likely cause of the vitellogenin induction (female egg yolk protein) in male or immature fish was the presence of natural estrogens such as estradiol and estrone, and the synthetic estrogen ethinyl-estradiol (used in birth control pills) (Brighty 1997; Giesy and Snyder 1998). Further studies demonstrated a clear relationship between concentration of sewage effluent and the percentage of intersex fish (male fish with eggs present in testicular tissue) (Jobling et al. 1998).

Environment Canada's scientists along with scientists from the Canadian Network of Toxicology Centres (CNTC) have been evaluating the Canadian environment for effects of sewage discharges on fish populations. To date, although some induction of vitellogenin in caged immature fish has been demonstrated downstream of some municipal effluent discharges, they have failed to replicate the high levels of vitellogenin induction as reported for fish in the United Kingdom (Servos et al. 1999). This was not unexpected, however, as the dilution of effluents in Canadian waters is often much greater than that found in Europe. Chemical analysis has also been conducted on effluents from a wide range of sewage treatment plants across Canada. Detectable levels of estradiol, estrone and ethinyl-estradiol, all identified in the United Kingdom studies (Brighty 1997), have also been found in effluents from Canadian sewage treatment plants (Burnison et al. 2000; Servos et al. 2000b). Other studies have been initiated on fish populations within the Fraser River through the Fraser River Action Plan and the Georgia Basin Ecosystem Initiative. Preliminary data indicate that male peamouth chub collected in the estuary have elevated vitellogenin levels two to four times background (Gray 1997). Researchers at Centre St-Laurent in Quebec also have preliminary evidence of hermaphrodism in some fish species collected in the St-Nicolas area of the St. Lawrence River near Quebec City and some vitellogenin induction in the hemolymph of clams from intertidal stations of the Saguenay fjord, Quebec (Blaise 1997). Other researchers through the CNTC have initiated a study examining the impact of Montreal municipal effluent on the immunological resistance in spottail shiner. Preliminary results suggest that immature fish downstream of the discharge have induced vitellogenin and stimulated immune systems (Cyr et al. 1999).

Environment Canada has also conducted preliminary studies examining the receiving waters downstream of a textile mill effluent. Caged fish exposed to effluents have elevated vitellogenin titres; however, this textile effluent is treated first in a municipal sewage treatment system, making it difficult to separate out the responsible sources (Servos et al. 2000b).

## Heavy Metals and Alterations in Adrenal Function

Dr. Alice Hontela at the University of Quebec at Montreal and other colleagues through the CNTC have identified the adrenal system of fish as one of the endocrine targets of environmental xenobiotics. Although these studies have not been utilizing the endpoints traditionally thought of as endocrine (estrogenicity), they have demonstrated a direct link between exposure and altered endocrine function. The acute toxicity of heavy metals is well documented; however, few studies have investigated the effects of chronic metal exposure on fish in their natural habitat. To assess the effects of heavy metal exposure, they have been investigating yellow perch (*Perca flavescens)* from lakes situated along a contamination gradient of zinc, copper, cadmium and iron in the Abitibi mining region. Fish exhibit an exposure-related decrease in condition factor, growth efficiency, and a reduced capacity to elevate blood cortisol, a metabolic hormone, in response to a standardized stress (Hontela et al. 1992). They have also developed new methodologies to assess the functional integrity of the steroidogenic adrenal/interrenal system of fish and have demonstrated reductions in gonadal development and circulating 17β-estradiol levels (Hontela et al. 1999).

## Pesticides, Insecticides and Agricultural Runoff

A recent study has found significant relationships between historical applications of an insecticide containing 4-nonylphenol (4-NP) and catch data for Atlantic salmon (*Salmo salar*) populations. The motivation to pursue the possible effects of Matacil 1.8D on fish came from making the connection between reported estrogenic effects of concentrations of 4-NP in exposures of trout in studies from the United Kingdom and knowledge of the formulation and concentrations of aminocarb present in surface waters after operational spraying in New Brunswick in the 1970s and 1980s (Fairchild et al. 1999a). When data for areas in which heavy spraying were compared to salmon catch data, there was a significant negative relationship between the returns of salmon and the proportion of the tributaries sprayed. Researchers suggested a possible link of these declines in catch data to effects of 4-NP on the parr-smolt transformation stage of fish development when the fish are becoming physiologically adapted for life in seawater. To test the hypothesis that 4-NP impairs parr-smolt transformation, Fairchild et al. (1999b) exposed Atlantic salmon smolts to environmentally relevant, pulse doses of waterborne 4-NP. To determine whether 4-NP was operating via its properties as a weak estrogen, smolts were also exposed to sustained doses of estradiol. The smolts capability to withstand seawater and their subsequent growth and survival were evaluated after exposure. Subsequent growth and survival in seawater was impaired from the various treatments above that of the control fish. If the effects exerted by 4-NP were due to its estrogenic potential, then estrogenic activity stemming from other sources (e.g., domestic sewage, agricultural wastes or phytoestrogens from pulp mills) might influence present-day salmon populations (Fairchild et al. 1999a). To address this question, the group has begun caging studies in a number of the locations to determine whether present-day runoff is affecting this important stage of fish development. Preliminary results suggest that reductions in smolt growth are occurring in areas of known agricultural input.

Following the 1997 EC workshop in which it was recommended that priority should be given to assessing those sites or sectors which have been identified as having the highest potential for adverse effects, studies were initiated to examine animal wastes and other agricultural runoff as potential endocrine disruptors in the Canadian aquatic environment. Preliminary studies were conducted examining large hog operations in collaboration with Agriculture Canada, which identified a number of locations for potential effects. Caging studies were conducted at a number of locations upstream and downstream of fields being sprayed with liquid pig manure. Although problems were encountered with the samples for vitellogenin analysis, detectable levels of estrogenic compounds were identified in the streams downstream of the manure source (Hartman et al. 1999). Studies are continuing to follow up preliminary results at the initial sites and potential other sites are being identified in collaboration with Agriculture Canada.

A large collaborative project has also been initiated to examine the effects of potato pesticides on aquatic life in the receiving environment in Prince Edward Island. Studies on wild fish in this area are difficult, however, as the majority of the waterways are relatively small with few resident fish species. The most dominant species present on the island are brook trout, and results from our initial wild fish collections showed no differences in circulating steroid hormone levels in fish between highly exposed and reference populations, and there was no evidence of the intersex condition in sacrificed fish (Gray et al. 2000). Studies are continuing using caged fish at a number of locations examining immature rainbow trout for evidence of vitellogenin induction and alterations in circulating steroid hormone levels (Sherry et al. 2000). Preliminary studies have also been initiated examining wild fish populations downstream of numerous agricultural areas in Southern Ontario for the presence of the intersex condition. Preliminary examination of the data suggests that very little evidence of the intersex condition has been found (McMaster et al. 1999). Further studies are examining fish for evidence of alterations in gonadal development at these sites as well as the examination of the ability of gonadal tissue to produce steroid hormones in vitro.

A large study has been initiated developing an effects-based cumulative effects framework for the freshwater component of the Saint John River in New Brunswick. The project includes the identification of priority areas of concern based on the characterization of fish performance in various reaches of the upper Saint John River basin, including areas potentially impacted by poultry and potato processing plants, other agricultural inputs and sewage effluents. These studies are also addressing the use of small forage fish species for reproductive evaluations.

Preliminary results suggest that some reproductive and growth alterations may be present in some of the resident fish populations (Gray and Munkittrick 2000; Munkittrick et al. 2000).

In the Lower Fraser Valley of British Columbia, a study is being conducted to assess the effects of stream waters affected by both agricultural and urban runoff on resident fish and crayfish species. Fish and crayfish are exposed to the stream water using stream-side aquariums supplied with flow-through stream water. Endpoints being assessed include induction of vitellogenin and the intersex condition as well as effects on egg development, hatch and larval survival.

## Refinery Discharges and Steel Mill Effluents

Studies have been conducted at a number of locations downstream of refinery discharges in Canada. Attempts to conduct wild fish surveys were made at some locations, but difficulties in reference site selection and other confounding factors were present. Laboratory studies with effluents have demonstrated induction of vitellogenin, and further caging studies are being conducted at some sites. Extensive caging studies have also been conducted in Hamilton Harbour to examine a number of endocrine parameters in fish exposed to steel mill effluents and municipal wastes. Results are preliminary; however, some induction of vitellogenin has been reported and modulation of a number of immune parameters was found (Sherry 2001).

Another recommendation from the Northern Rivers Basin Study was that research should be undertaken to identify the effects on aquatic biota of exposure to substances arising from the oil sands, both naturally and as a result of oil sands industry development (Canada-Alberta-Northwest Territories 1997). Previous studies have demonstrated significant reductions in reproductive sex steroid hormone levels in yellow perch exposed to oil sands refinery waste water (van den Heuvel et al. 1999). Studies have now begun on both large and small-bodied sentinel species in the area to establish existing impacts and to establish background data on fish populations to monitor further development. Forage fish species are also being used to attempt and separate out the effects of both natural and anthropogenic sources of PAHs on fish reproductive function (Tetreault et al. 2000).

## Tributyltin

Tributyltin (TBT) has been found in sediments, water and biota of coastal areas and its source is primarily due to leaching from marine paints containing TBT. Although there are now regulations that prohibit the use of TBT in many countries (especially on smaller boats), detectable levels are still found in aquatic organisms (Fent 1996). In the early 1980s, it was reported that the antifouling agent TBT was effective in producing a condition termed "imposex" in a marine snail species. Imposex, which is the imposition of male sex organs (penis, vas deferens) on female organisms, was subsequently confirmed in a number of other marine gastropod species exposed to TBT. Recent studies have identified imposex in dogwhelks (*Nucella lapillus*) on both the Atlantic and Pacific coasts of Canada. In a preliminary survey of gastropods at Passamaquoddy Bay, St. Andrews, New Brunswick, in 1998, snails were collected during low tide and cracked open, and the sex determined by gross morphological observation. Females possess a distinct sperm-ingesting gland while males have a penis on the right side of their body. Females with a penis were considered to demonstrate imposex, and 6.5% of the collected dogwelks showed this condition (Currie et al. 2000). Tissues were also processed histologically, and microscopic observation indicated changes within the capsule gland and ovaries as well as imposition of penile tissue and vas deferens over the oviduct of imposexed females. Testicular tissue (seminiferous tubules) developed in some of the examined imposexed specimens (Currie et al. 2000). Although this level of imposex corresponds to other sites around the world, it was lower than in samples collected at this site in 1969 when the incidence rate was 15.1%. It is thought that TBT exposure results in feminization of male gastropods through effects on the endocrine system as TBT increases testosterone levels, likely through an inhibition of the aromatase enzyme, which converts testosterone to 17β-estradiol. Higher testosterone levels result in the development of a penis in females or the imposex condition (Fent 1996). Very few studies have been conducted on the effects of TBT on fish populations. Laboratory studies suggest that TBT may have direct effects on the young through the maternal transfer to the eggs. Effects have been demonstrated on egg number, fertilization success, percent hatch and survival of swim up larvae (Nirmala et al. 1999).

## Conclusions

Early Canadian studies on fish, particularly those in the Great Lakes, have been critical in bringing the endocrine disruptor issue to the forefront. It is clear that Canada's strength lies in the breadth and depth of its field research programs. It is this world-recognized expertise and strength that Canada can contribute to the international effort to address this issue. Following the workshop that was held to establish a national agenda for the scientific assessment of endocrine disrupting substances (Servos et al. 2000a), a number of conclusions were made regarding endocrine disrupting substances in Canadian aquatic ecosystems. It was clear that effects have been detected at some sites in Canada (Table 1) and that there were additional sites that have not yet been investigated. Some of the effects identified may not occur directly through an endocrine mechanism, or in some cases the mechanism is not yet known. However, this does not diminish the importance of the effects or observations. Rather than concentrate on specific modes or mechanisms of action such as endocrine disruption, it was recommended that Canada should focus on sublethal effects on growth, reproduction and developmental endpoints in aquatic ecosystems. The major sites and or sectors of concern for the Canadian aquatic ecosystem were identified as presented in Table 2.

**Table 1.** Endocrine-related responses identified in fish and other aquatic organisms within the Canadian Environment

| Source, sector, location or species | Identified compounds | Response measured | Endocrine disruption? | References |
|---|---|---|---|---|
| Great Lakes salmonids | TCDD and related compounds | Larval mortality | Potential | Cook et al. 1994, 1997 |
| | Unknown | Thyroid abnormalities | Potential | Leatherland 1993 |
| Pulp and paper mill effluent | Unknown | Altered reproductive function | Yes | McMaster et al. 1991<br>Munkittrick et al. 1991<br>Van Der Kraak et al. 1992 |
| Municipal wastes | Estradiol, estrone and ethinylestradiol | Vitellogenin induction | Potential | Servos et al. 2000b<br>Gray 1997<br>Blaise 1997<br>Cyr et al. 1999 |
| | Unknown | Immune stimulation | Potential | Cyr et al. 1999 |
| Heavy metals | Zinc, copper, cadmium, iron | Altered adrenal function | Yes | Hontela et al. 1992 |
| Pesticides, insecticides and agricultural runoff | 4-NP | Altered smoltification | Yes | Fairchild et al. 1999ab |
| | Equol, estradiol | Endocrine active compounds present | Potential | Hartman et al. 1999 |
| | Unknown | Reproduction and growth | Potential | Munkittrick and Gray 2000<br>Munkittrick et al. 2000 |
| Refinery discharges and steel mill effluents | Estradiol, estrone and ethinylestradiol | Vitellogenin induction | Potential | Sherry 2001 |
| | PAHs | Immune modulation | Potential | Sherry 2001 |
| | PAHs | Sex steroids | Potential | Van den Heuvel et al. 1999<br>Tetreault et al. 2000 |
| Tributyltin | TBT | Imposex | Yes | Currie et al. 2000 |

**Table 2.** Major Canadian sites and/or sectors of concern for potential endocrine disruption in fish (Servos et al. 2000a)

| | Site or sector identified |
|---|---|
| 1 | Municipal effluents |
| 2 | Intensive agriculture, including pesticides and livestock production |
| 3 | Textile mill effluents |
| 4 | Pulp and paper sector |
| 5 | Mining and metals |
| 6 | Historically contaminated sites, e.g., Sydney Tar Ponds |
| 7 | Identified Areas of Concern, e.g., Great Lakes AOCs |
| 8 | Contaminants in the Arctic, including aboriginal foods |

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