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An Overview of Substances Present in Canadian Aquatic Environments Associated with Endocrine Disruption

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Numerous environmental contaminants have been associated with the ability to affect the endocrine status of animals and with the potential to elicit effects on individuals or populations in Canadian aquatic environments. Potential endocrine disrupting substances (EDS) consist of almost every class of environmental contaminants reported to date, including industrial chemicals, historical and current use pesticides, metals, and different classes of natural products. It has been difficult to establish cause-and-effect relationships with potential EDS for several reasons: i) the diversity of ways that chemicals can influence endocrine systems challenges efforts to characterize chemicals that can cause endocrine responses, ii) many responses in aquatic biota have been associated with complex mixtures where the causative agents remain unidentified, and iii) most literature information deals with mammalian studies using pure compounds so there is considerable uncertainty regarding extrapolation to aquatic species and efficacy of environmental concentrations. An overview of the literature on EDS, specific to exposure within Canadian aquatic environments, is presented to emphasize the diversity and complexity of chemicals capable of altering endocrine function.

Key words: **endocrine disrupting substances, aquatic ecosystems, pesticides, dioxins and furans, industrial chemicals, natural products**

Introduction

Endocrine systems involve complex mechanisms that coordinate and regulate internal communication among cells. Secretory glands release hormones that act as chemical messengers that interact with receptors in target cells to trigger specific responses such as induction of hormone-responsive genes. Endocrine systems can be affected by a wide variety of chemicals, including environmental contaminants, which can potentially lead to an adverse effect on the organism. Even very subtle effects on endocrine function, particularly at critical developmental periods can result in changes that may influence growth, reproduction or behaviour, and may affect subsequent generations.

Endocrine disrupting substances (EDS) have often been associated with those chemicals that have the potential to bind to the estrogen receptor, lead-

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ing to the mimicking of effects mediated by endogenous hormone. However, this represents only one mechanism by which chemicals can modulate or disrupt the normal function of endocrine systems. Chemicals can disrupt a wide variety of hormonal processes, including hormone synthesis and metabolism, without directly acting through a receptor mechanism (Van Der Kraak et al. 1998; National Research Council 1999). Chemicals with the ability to influence endocrine function are therefore diverse and many definitions have been proposed by a variety of scientific and governing bodies (e.g., U.S. EPA 1997). For the purposes of this overview, the *Canadian Environmental Protection Act, 1999,* definition of a hormone disrupting substance (CEPA 1999, article 3, subsection 43) is applied:

> *"Hormone disrupting substance" means a substance has the ability to disrupt the synthesis, secretion, transport, binding, action or elimination of hormones in an organism, or its progeny, that is responsible for the maintenance of homeostasis, reproduction, development or behaviour of an organism.*

Here, EDS are recognized to include specific chemicals as well as mixtures and effluents, where the active chemicals are not necessarily known. This is especially relevant to Canadian aquatic environments where a variety of hormone-like responses have been observed in aquatic biota exposed to complex mixtures or effluents (reviewed in McMaster 2001, this issue). It must be emphasized that most of the individual chemicals discussed in this overview, while reported in Canadian environments, are not necessarily causing adverse effects on Canadian aquatic biota or ecosystems. The intent of this overview is to identify and characterize the wide variety of substances in the Canadian environment with the potential to affect the endocrine function of aquatic biota. For this discussion, specific chemicals associated with endocrine disruption have been categorized as industrial chemicals, natural products, pesticides, dioxins and furans and other environmental pollutants (Table 1). An additional discussion of the contributions of known and unidentified EDS to effects associated with complex mixtures is also given (Table 2).

EDS: Old and New

Considerable research on the exposure of organisms to environmental contaminants has focused on persistent, bioaccumulative toxic substances (PBTs) and persistent organic pollutants (POPs). PBTs and POPs can move through food chains and represent a hazard to top predators in the ecosystem, including fish-eating birds and mammals. These substances include a wide variety of industrial chemicals and by-products such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/DFs) as well as organochlorine pesticides. Many of PBTs and POPs continue to contaminate Canadian aquatic ecosystems and have been distributed throughout the globe through atmospheric transport. In remote areas of the Canadian Arctic, elevated levels of these compounds have been detected in wildlife (Department of Fisheries and Oceans 1998; Braune et al. 1999; Muir et al. 1999; Macdonald

Table 1. Major chemical classes associated with endocrine disruption and their inputs to Canadian aquatic environments

Class	Examples of specific chemicals	Inputs to Canadian aquatic environments	Selected references
Industrial chemicals	PCBs + metabolites	• Industrialized harbours • Atmospheric deposition	Delzell et al. 1994 Bergeron et al. 1994 Leatherland and Sonstegard 1978 Guillette and Crain 1996
	Non-ionic surfactants (APEs)	• Municipal sewage effluents • Textile effluents • Industrial sites	Bennie 1999 Servos et al. 2000 Fairchild et al. 1999
	Brominated diphenyl esther	• Industrialized harbours • Flame retardant leachates	Luross et al. 1999 Fowles et al. 1994
	Phthalates	• Plastic leachates	Lee et al. 2000
	Bisphenol A	• Municipal sewage • Industrialized harbours	Ikonomou et al. 2000 Zacharewski et al. 1998
Pesticides	Organochlorines (e.g., DDT)	• Atmospheric deposition • Agricultural runoff	Huestis et al. 1996 Braune et al. 1999 Muir et al. 1999 Macdonald et al. 2000

(continued)

Table 1. *(continued)*

Class	Examples of specific chemicals	Inputs to Canadian aquatic environments	Selected references
Halogenated organic contaminants	TBT	• Marine harbours • Application sites	Maguire 1999
	PCDD/DFs	• Industrialized harbours • Wood preservatives • Contaminated sediments	Huestis et al. 1996 Muir and Patershank 1996 Walker and Peterson 1991
Other pollutants	Heavy metals	• Acid rain • Chlor-alkali plants • Mine tailings	Braune et al. 1999 Brodeur et al. 1997
	PAHs	• Industrialized harbours • Atmospheric deposition • Oil sands deposits and processing	Munkittrick et al. 1995 van dan Heuvel et al. 1999 Tran et al. 1996 Lockhart et al. 1992
Natural products	17β-estradiol	• Municipal sewage • Animas waste runoff	Lee et al. 2000 Ternes et al. 1999
	Plant sterols Stillbenes Flavonoids	• Pulp mill effluents • Agricultural runoff	Burnison et al. 2000 Dodge 1998 Safe and Gaido 1998

et al. 2000). PBTs and POPs therefore remain a concern for the environment even though their use and production in Canada has been strictly controlled or banned.

Recent attention has been focused on a variety of chemicals that do not fall into the category of PBTs or POPs, yet have the potential to affect the growth and development of aquatic biota by altering endocrine function. This includes chemicals commonly found in industrial or municipal effluents as well as urban or agricultural runoff. Aquatic biota may be exposed to these materials during critical periods of development which could lead to irreversible impacts. Several chemicals with the potential to affect endocrine function have been identified, and chemical-specific assessments have been initiated in Canada (e.g., nonylphenol and its ethoxylates and tributyltin (Servos et al. 1999; Maguire 2000). It is important to note that most of these chemicals exist within complex mixtures where additional causative agents are difficult to isolate and identify. The polarity and high water solubility of chemicals within this category represent a departure from traditional environmental chemistry and provide a challenge to develop analytical methods to determine exposures and persistence in the environment.

Categories of Chemicals Functioning as EDS

Industrial Chemicals

Historically, much attention has been given to industrial chemicals linked to effects on growth, reproduction and development (Fig. 1). PBT industrial chemicals, typically organochlorines, are found throughout Canadian aquatic environments, sediments and biota (Department of Fisheries and Oceans 1998; National Research Council 1999). While higher concentrations are generally found near industrialized centres, atmospheric transport and polar condensation have led to the detection of many of these chemicals in arctic regions (Department of Fisheries and Oceans 1998; Braune et al. 1999; Macdonald et al. 2000; Muir et al. 1999). One of the most well-studied classes of industrial chemicals is the family of PCBs. PCB levels reached peak environmental concentrations in sediments and aquatic biota in the 1970s, have declined in many Canadian ecosystems such as the Great Lakes (Department of Fisheries and Oceans 1998, Huestis et al.,1996), and the Fraser River Basin (Macdonald et al. 1998), and leveled off during the late 1980s to early 1990s. However, in some areas, fish tissue residues remain a concern for human consumption (Macdonald et al. 1998). Current inputs to Canadian ecosystems are a concern in the Arctic, where there is little evidence of reduced PCB loadings (Macdonald et al. 2000). PCB inputs to the Arctic and temperate regions occur primarily through aerial deposition, where there is a fractionation toward more volatile lower-chlorinated compounds. Despite global reductions in the manufacture and use of PCBs, it is anticipated that corresponding reductions in Arctic concentrations will be not occur for several decades (Macdonald et al. 2000).

PCBs and their hydroxylated metabolites have been linked to several endocrine-related effects in aquatic species, such as early life stage mortality in fish (Delzell et al. 1994), sex reversal in turtle embryos (Bergeron et al. 1994) and impaired thyroid function in fish (Leatherland and Sonstegard 1978) through altered binding of thyroid hormone to plasma proteins (National Research Council 1999), but their exact role in endocrine responses observed in regions such as the Great Lakes are difficult to separate from other factors, such as dietary deficiencies.

Phthalate esters have been widely used for decades as plasticizers, more recently as pesticide inert ingredients, and as replacements for PCBs (Table 1). Depending on esterification, these compounds span a wide range of hydrophobicity (log K_{ow} >2–8), compete with varying degrees for mammalian estrogen receptors and display weak in vitro estrogenic activity (Zacharewski et al. 1998). Phthalates are ubiquitous in the environment and can be problematic from an analytical standpoint because of their background presence. As such, concentrations are not regularly reported; however new instrumental techniques such as LC-MS show promise for measurement in environmental matrices (Ikonomou et al. 2000).

There has been recent concern over the activity of polybrominated diphenyl ethers (PBDPEs [Fig. 1]), which are used in flame retardants. PBDPEs are persistent, lipophilic compounds that have been shown to affect immune function (Fowles et al. 1994) and may mimic the action of thyroid hormones. There is currently little information on levels in Canadian ecosystems, but recent analytical method development studies have reported these compounds in Lake Ontario lake trout (*Salvelinus namaycush*), sockeye salmon (*Oncorhynchus nerka*) and pacific herring (*Clupea pallasii*) (Luross et al. 1999).

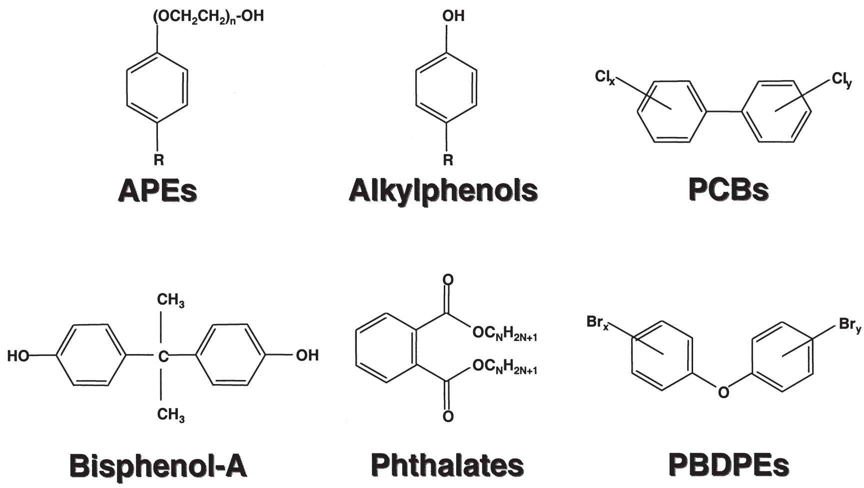


Fig. 1. Examples of industrial chemicals associated with endocrine disruption: alkylphenol ethoxylates (APEs), phthalates; polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDPEs) and bisphenol-A.

Bisphenol-A is used industrially as a chemical intermediate in polymers, resins, dyes and flame retardants. It is one of the top 50 chemicals produced in the U.S., with over 1.6 billion pounds produced in 1995 (National Research Council 1999). Concern over this chemical originated from the discovery that leachates from plastic coatings inside food and beverage cans were estrogenic (Soto 1992). Mammalian studies have shown conflicting responses, but potential estrogenic effects are possible within the range of human exposure (National Research Council 1999). Studies have shown that this compound does leach from plastic coatings within food and beverage cans and is found in saliva from dental patients. Bisphenol A has a log K_{OW} of 3.3 (Hansch and Leo 1981) and an estimated bioconcentration factor of 10–100 (Kawasaki 1980). While these properties render bisphenol-A bioavailable and it has been detected in Canadian municipal sewage effluents (Lee et al. 2000), it is readily degraded in activated sludge treatment systems and has a half-life of 1–4 days in ambient waters (Staples et al. 1998). At this time, it is not known if environmental exposures affect endocrine status in aquatic biota.

Recently, the nonionic alkylphenol ethoxylates (APEs) and alkyl phenol degradation products (Fig. 1) cause estrogenic activity in vitro and have been implicated as partially responsible for induction of the circulating egg yolk protein vitellogenin (Vg) and intersex (oocytes in testes) in fish exposed to municipal sewage effluents in the U.K. (Desbrow et al. 1998). In Canada, experiments with final municipal sewage effluents have shown weak Vg induction in rainbow trout caged immediately downstream of outfalls; alkylphenols and APEs account for a small proportion of the total effluent estrogenic activity in vitro (Servos et al. 1998a; Burnison et al. 2000). There remains uncertainty regarding the potential estrogenic potency of nonylphenol, nonylphenol ethoxylates and nonylphenol ethoxycarboxylates (Servos et al. 1999). The distribution of these compounds varies considerably depending on the type and degree of effluent treatment, such that some effluents may contain concentrations of the metabolites in final effluent that may result in estrogenic responses in fish in the receiving environment (Servos et al. 2000). APE loadings range from <1 to <200 µg/L alkylphenols and <1 to <150 µg/L ethoxylates from Canadian sewage treatment plants (Bennie 1999). Sewage sludges and effluents from textile facilities may contain higher concentrations of these materials depending on the use and treatment processes. Alkylphenols have low to moderate bioaccumulation potential and APEs are not expected to bioaccumulate significantly (Servos 1999). APEs are not persistent in aquatic environments, but their degradation products, including alkylphenols, are moderately persistent in anaerobic sediments and found in aquatic sediments (Maguire 1999; Bennie 1999; Servos et al. 2000).

A significant relationship has been established between the timing of historical insecticide applications containing 4-nonylphenol as an adjuvant in Atlantic Canada and impairment of smoltification of Atlantic salmon (*Salmo salar*) (Fairchild et al. 1999). Although 4-nonylphenol is no longer used in this way, potential exposure of Atlantic salmon to alkylphenols in municipal effluents during the sensitive smoltification process is possible.

Pesticides

Several organochlorine (OC) pesticides that have been historically associated with reproductive effects in wildlife are persistent and bioaccumulate in aquatic food webs (Fig. 2). Global distribution from continued use in other countries contributes to the seasonal variations in deposition from the atmosphere. Atmospheric deposition represents the primary input of many of these chemicals to Canadian aquatic systems, so further declines in the levels of these chemicals and other PBTs are not expected to be as drastic as in the last decade. In the Arctic, the rapid movement of POPs and PBTs deposited in snowfall occurs during spring snowmelt and results in transfer to the marine ecosystem, which is then combined with inputs associated with marine currents (Muir et al. 1999). As a result, the Arctic Ocean has become a major reservoir for semi-volatile organochlorine pesticides such as the hexachlorocyclohexanes (Macdonald et al. 2000).

Many of the OC pesticides bind to the estrogen receptor and can lead to measurable responses in vitro, e.g., cell proliferation in human breast cancer cells (Preziosi 1998) and induction of vitellogenin in frogs by DDT (Palmer and Palmer 1995). While much has been made of the in vitro activity associated with OC pesticides, it has been difficult to link recovery in aquatic biota (Heinz 1998) to the decreased levels of these contaminants that have been observed in Canadian aquatic ecosystems (Department of Fisheries and Oceans 1998; Macdonald et al. 1998). The most extensive historical database on effects and contaminant levels exists for the Great Lakes and while it is plausible that DDT could have caused reproductive impairment of salmon populations based on historical concentrations in fish eggs, this has not been definitively established (National Research Council 1999). Similarly, elevated concentrations of DDT, DDE and dieldrin in snapping turtle (*Chlydra serpentina*) eggs in the Great Lakes and the upper St. Lawrence River have been correlated with embryonic mortality and deformities (Bishop et al. 1991) but direct causal linkages have not been found. Sex reversal and intersex can be induced in turtles by selected hydroxylated PCB metabolites but these isomers are not typically produced by aquatic biota (Guillette and Crain 1996).

Vinclozolin is a dicarboximide nonsystemic fungicide that is used for the control of several types of fungi in vines, strawberries, vegetables, fruit and turf grass (Fig. 2). Concern has arisen from studies that have demonstrated the developmental anti-androgenic activity of vinclozolin and its metabolites in mammals (Kelce et al. 1994). There is some uncertainty regarding the degree of aquatic exposures. Vinclozolin is of low to moderate persistence in soil, with estimated half-lives ranging from 3 days to greater than 3 weeks (U.S. EPA 1991). Photolysis and hydrolysis may occur, and are pH dependent, with greater photolysis and hydrolysis under neutral or slightly basic conditions (U.S. EPA 1991). With a log K_{ow} of approximately 3, this compound would be bioavailable to aquatic biota, at the present time it is not known if exposures are sufficient to cause effects.

Atrazine is a selective triazine herbicide used to control broadleaf and grassy weeds in corn, Christmas trees and other crops, and in conifer reforestation plantings (Fig. 2). Atrazine has been associated with an increased testosterone metabolism (aromatase activity) in rats (reviewed in Preziosi 1998) and altered steroidogenesis in alligators dosed *in ovo* (Crain et al. 1997). Atrazine is used more than any other herbicide with 31,000 tonnes applied annually to soils in the U.S. Depending on soil type, atrazine can be highly persistent in soil. Chemical hydrolysis, followed by degradation by soil microorganisms, is the primary degradation pathway of atrazine (Briggs 1992). Hydrolys is rapid in acidic or basic environments, but is slower at neutral pH. Addition of organic material increases the rate of hydrolysis. Atrazine can persist for longer than 1 year under dry or cold conditions (Howard 1989). Because it does not adsorb strongly to soil particles and has a lengthy half-life (60 to >100 days), it has a high potential for movement into ground and surface waters (Wauchope et al. 1992). In 1991, Health Canada eliminated autumn applications and reduced the maximum spring applications to corn to reduce levels detected in spring runoff. Today, atrazine is frequently detected in Canadian surface waters draining application areas in the low mg/L range; a recent risk assessment predicts no acute effects to aquatic life at these levels (Solomon et al. 1996) but it has not been established if endocrine-related effects on aquatic organisms are occurring.

The use of organotin compounds, particularly tributyltin (TBT [Fig. 2]) as biocides in paints used to protect aquaculture nets and ship hulls was restricted in 1989 to vessels >25 m in length. These compounds have been shown to cause imposex in common female whelks (*Buccinum undatum*) (reviewed in Maguire 2000). Highest concentrations are localized around biocidal application areas and their persistence depends on light,

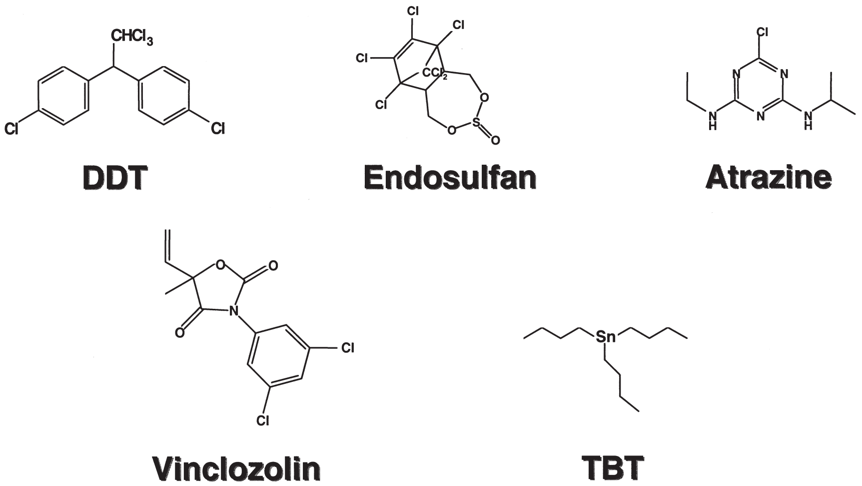


Fig. 2. Examples of organochlorine pesticides associated with endocrine disruption: organochlorine pesticides DDT and endosulfan, atrazine, vinclozolin and tributyltin (TBT).

temperature and oxygen availability (Department of Fisheries and Oceans 1998; Maguire 2000). The use of TBT is expected to be eliminated in Canada by 2003.

Modern pesticides are designed to rapidly degrade in the environment, reducing their persistence. For modern pesticides, there is a general lack of information relating laboratory responses to field effects, particularly effects related to residues found in Canadian waterways. A detailed evaluation of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) has shown that laboratory responses may not be manifested in field situations. Field formulations of TFM were found to rapidly induce mixed function oxygenase (MFO) activity and affect circulating steroids in fish (Hewitt et al. 1996). TFM itself was shown to induce Vg in vitro (Hewitt et al. 1998a); however, MFO induction was short lived and Vg was not observed following a field application (Hewitt et al. 1998b). The paucity of information on effects of modern pesticides has been identified as a research priority in Canada (Environment Canada 1997) and several research projects were initiated in high-use areas of Canada in the late 1990s to evaluate the potential for residues to affect endocrine function (see "Complex Mixtures and Effluents").

Dioxins and Furans

The environmental persistence, hydrophobicity, bioaccumulation, acute toxicity and effects on early development in fish associated with PCDD/DFs have been well documented (Walker and Peterson 1991; Delzell et al. 1994; Walker and Peterson 1994). PCDD/DFs have been designated as Track 1 substances for virtual elimination in Canada under the Toxic Substance Management Policy. The effects related to PCDD/DFs are isomer dependent and are directly related to the isomeric affinity for the cytosolic aryl hydrocarbon receptor (AhR). Historically, the major sources of PCDD/DFs to Canadian aquatic environments have been associated with effluents from wood pulping facilities with lesser contributions from municipal sewage and contamination of wood preservatives (Department of Fisheries and Oceans 1998; National Research Council 1999). With the exceptions of Lake Ontario and Lake Superior, concentrations in the Great Lakes have shown general declines of PCDD/DFs in top predator fish collected from the late 1970s to the early 1990s (Department of Fisheries and Oceans 1998). Industry-wide initiatives within the Canadian pulp and paper sector have reduced total organochlorine discharges and releases of PCDD/DFs have dropped substantially since the late 1980s (Department of Fisheries and Oceans 1998). A parallel decrease in tissue levels in fish captured in receiving waters in Ontario (Servos et al. 1997) and in Alberta (Muir and Pastershank 1996) have also been observed with the implementation of process changes, particularly the reduction in the use of molecular chlorine. The role of PCDD/DFs in reproductive dysfunction in wild fish exposed to pulp mill effluents remains as yet to be resolved, but evidence now indicates that additional chemicals are involved (Van Der Kraak et al. 1998; Hewitt et al. 2000).

While PCDD/DFs possess anti-estrogenic properties, they do not appear to elicit these responses through a pathway mediated directly by the estrogen receptor. Recent evidence indicates that anti-estrogenic effects are mediated through the AhR (Kharat and Saatcioglu 1996) with cross-talk to the estrogen receptor and a heat shock protein (Caruso et al. 1999). It therefore follows that other compounds, collectively acting through the AhR, may have historically contributed to AhR-mediated toxicity and endocrine responses observed in aquatic life. Although they have been declining, the current concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents in Lake trout fry in the Great Lakes are near the threshold for mortality (Walker and Peterson 1994).

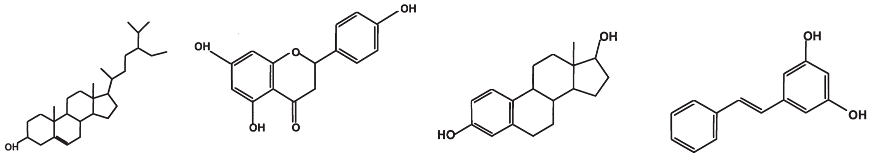
Other Environmental Pollutants

One of the relatively less-studied family of compounds associated with endocrine disruption are the polyaromatic hydrocarbons (PAHs). In Canada, the highest aquatic exposures to PAHs are localized in harbour sediments near urban and industrial centres as well as in proximity to naturally occurring bitumen deposits in western Canada. Atmospheric transport contributes to low-level loadings to other freshwater environments and the arctic (Lockhart et al. 1992). In areas where temporal trend information is available, concentrations of PAHs have decreased over the last several decades, largely through emission controls (Department of Fisheries and Oceans 1998). The environmental persistence of PAHs is primarily related to factors affecting their photodegradation. PAHs have been associated with anti-estrogenic activity in vitro (Tran et al. 1996) and effects on circulating steroids in rainbow trout (*Oncorhynchus mykiss*) (Munkittrick et al. 1995). PAHs are metabolized by most vertebrate species and metabolic formation of toxic products can occur (Di Giulio et al. 1995). Bioconcentration of parent PAHs are a concern for invertebrate species that possess limited metabolic capacities for these compounds. There is also uncertainty regarding the potential of PAH derivatives (alkyl, nitro) and more water-soluble, photomodified products to affect endocrine function in aquatic species. The effects of naturally occurring PAHs are currently being examined in fish exposed to bitumen deposits in western Canada. Results from initial studies using yellow perch (*Perca flavescens*) stocked in experimental ponds designed to emulate possible aquatic reclamation alternatives of oil sands showed little differences when compared to reference sites (van den Heuvel et al. 1999); however, napthenic acids contained in fractions of tailings pond water have been linked to deformities, early hatch and reduced survival in yellow perch and Japanese medaka eggs (Peters 1998).

Another class of environmental pollutants linked to endocrine responses is the heavy metals (cadmium, lead, mercury, selenium, copper and zinc). Heavy metals have been associated with impaired stress responses and adrenal function in fish in vitro (Brodeur et al. 1998) and in wild fish collected from contaminated lakes (Brodeur et al. 1997). Natural concentrations of heavy metals are generally considered too low to pose any threat to aquatic life but human activities have increased their concentrations in aquatic ecosystems. Acute toxicity, environmental persistence and bioaccumulation depend on several factors, including organometallic forms, pH, presence of dissolved organic carbon and hardness. Sediment accumulations of lead and other metals in eastern Canadian lakes are related to the pattern of acidic deposition (Department of Fisheries and Oceans 1998). Elevated concentrations of metals in marine ecosystems can be derived from precipitation from freshwater sources. Well-flushed coastal areas tend not to accumulate precipitated metals. Point sources include mining sites and chlor-alkali plants as well as sewage outfalls, and although there have been declines in recent decades, elevated concentrations still persist in some sediments (Department of Fisheries and Oceans 1998). Discharges from urban centers and atmospheric deposition have become important contributors of metals to Canadian aquatic ecosystems, particularly in the Arctic. In contrast to other metals, mercury has been increasing in some freshwater sediments and biota in recent years via atmospheric deposition (Department of Fisheries and Oceans 1998). A number of lakes in the Arctic and northern Quebec have fish populations with mercury concentrations frequently exceeding subsistence consumption guidelines of 0.2 mg/g (Braune et al. 1999).

Natural Products

Much recent attention has been directed toward natural products that can function as EDS (Fig. 3), particularly those derived from plant material and those contained within human and animal waste (see "Complex Mixtures"). Nearly 200 different plant compounds that vary highly in potency have been associated with estrogenic activity (Dodge 1998; Safe and Gaido 1998); however, with a few exceptions, exposures to aquatic biota are not significant. Most studies have documented estrogenic activity associated with flavonoids found in soy products, such as genestein, and its metabolite equol (Fig. 3). Equol has recently been identified as an estrogenic component present in animal waste runoff from hog farming operations (Hartmann et al. 2000; Burnison et al. 2000); the environmental loadings and fate of this compound are currently being investigated. Genistein in estrogenic in yeast assays shows the ability to cause intersex and feminization of male Japanese medaka (*Oryziaas latipes*) and has been measured in effluent from a bleached kraft mill in Ontario at low mg/L



Sterols **Genistein** **17β-estradiol** **Stilbenes**

Fig. 3. Examples of natural products associated with endocrine disruption: plant sterols, the flavonoid Genistein, 17β-estradiol and stilbenes.

concentrations (Kiparissis et al. 2000). b-sitosterol, the dominant plant sterol consistently measured in pulp mill effluents, can induce Vg production and reduce plasma sex steroids in vivo (MacLatchy and Van Der Kraak 1995; Tremblay and Van Der Kraak 1998) at effluent concentrations reported for Canadian mills. A phytosterol mixture at 20 mg/L has demonstrated the ability to affect egg mortality and hatching success in brown trout (*Salmo trutta lacustris*) (Lehtinen et al. 1999). Stilbenes, also present in pulp mill effluents, have been associated with mixed function oxygenase (MFO) induction (Burnison et al. 1999) and potential estrogenic effects (Mellanen et al. 1996) in fish (Fig. 3). However, the levels of these chemicals detected in final effluents do not explain the patterns of responses observed in wild fish (Hewitt et al. 1995; Van Der Kraak et al. 1998).

Complex Mixtures and Effluents

Evidence of endocrine-related effects at different levels of biological organization in aquatic ecosystems is provided by field and laboratory studies of aquatic biota exposed to complex mixtures (reviewed in McMaster 2001, this issue). For the majority of complex mixtures known to be affecting endocrine status in aquatic species, there are uncertainties regarding the contributions of known EDS present and the role of unidentified EDS (Table 2). Toxicity identification evaluation (TIE) studies use activity in bioassays to direct chemical fractionations of mixtures to isolate and characterize bioactive components. This approach, coupled with mechanistically linked endocrine related endpoints, has shown potential in establishing the relative contributions of known and previously unidentified EDS. Isolating and characterizing the responsible chemicals in environmental situations will lead to enhanced risk assessments, risk management and mitigation efforts.

Effluents from Pulp and Paper Mills

One of the most well documented examples of EDS in Canada relates to effects of pulp mill effluents on wild fish reproduction. Laboratory and mammalian studies over the past decade have determined that certain chemicals in final effluents (PCDD/DFs, phytoestrogens, stilbenes [Table 2]) individually have the potential to affect endocrine homeostasis. Industry-wide process changes implemented by the mid 1990s have drastically reduced PCDD/DF discharges, and parallel reductions in fish tissues in Canada have followed (Servos et al. 1997). Although these process changes have resulted in improvements in reproductive performance of wild fish in some sites, persistent responses continue to be observed at several sites (reviewed in McMaster 2001, this issue). The pattern of responses on hormone levels in fish indicates that the causative agents are not persistent and bioaccumulative, rather, continuous exposure is required. Considerable attention in this regard has been directed to the actions of natural products detected in effluents. Stilbenes, lignans and plant sterols, particularly sitosterol, can be present in significant quantities in effluent

Table 2. Complex mixtures in Canadian aquatic environments known or suspected to have the potential to affect endocrine function in aquatic biota

Complex mixture	Potentially associated chemicals	Selected references
Pulp and paper mill effluents	Natural wood derivatives PCDD/DFs Plant sterols	Burnison et al. 1999 Hewitt et al. 2000 Van Der Kraak et al. 1998 MacLatchy et al. 1995
Municipal effluents and urban runoff	APEs Endogenous and synthetic hormones Bisphenol-A PAHs	Servos et al. 2000 Lee et al. 2000
Textile mill effluents	APEs	Servos et al. 2000
Contaminated sediments	PAHs PCBs PCDD/DFs Heavy metals	Munkittrick et al. 1995 Macdonald et al. 1998
Agricultural runoff	Pesticides	Gray et al. 2000 Hewitt et al. 1999a,b
	Endogenous hormones	Teather et al. 2001
	Phytoestrogen metabolites	Burnison et al. 2000

discharges and have exhibited estrogenic activity in laboratory studies (MacLatchy et al. 1995; Van Der Kraak et al. 1998). Difficulties in extrapolating from the concentrations of test chemicals and species used in laboratory studies to actual exposure situations have hindered efforts to establish linkages between the properties of these chemicals and wild fish responses. At present, the concentrations of chemicals demonstrating activity individually in laboratory studies do not completely explain the responses observed, making it difficult to pinpoint the mechanisms involved. Recent bioavailability studies have determined that fish exposed for a short term to effluent accumulate multiple chemicals that interact with sex steroid receptors, potentially affecting sex hormone signaling and transport (Hewitt et al. 2000). While bioavailability undoubtedly plays a role in the responses, the pulp mill effluent example also illustrates the need to consider competing and antagonistic interactions in cumulative responses of complex mixtures. For example, the cumulative estrogenic response associated with effluent constituents could be affected by androgenic activity associated with plant sterol transformation products (Howell and Denton 1989), and antiestrogenic activity exhibited by unidentified Ah receptor agonists (Zacharewski et al. 1995).

Effluent from Municipal Sewage Treatment Plants

Jobling et al. (1998) has documented that up to 100% of male roach collected downstream of sewage outfalls in England exhibited intersex. Previous studies documented large increases in Vg in male fish caged downstream of sewage outfalls in England (Prudom et al. 1994). The causative agent was originally suspected to be alkylphenolics. Alkylphenolics are common and often abundant contaminants in municipal effluents and are known to cause intersex, Vg induction, reduced gonad growth and other endocrine-related effects in fish (reviewed in Nimrod and Benson 1996; Servos 1999). Recent fractionation experiments indicate natural and synthetic estrogens (17β-estradiol, estrone 17α-ethynylestradiol) (Desbrow et al. 1998; Routledge et al. 1998) are associated with estrogenic responses. Similar studies in southern Ontario confirm that Canadian effluents are capable of causing Vg induction in immature trout (Servos et al. 1998a; Gamble et al. 1998). Bioassay-directed fractionations of Canadian effluents confirm that the majority of estrogenic activity is associated with natural and synthetic estrogens and lower activity is potentially associated with alkylphenols (Burnison et al. 2000). Natural and synthetic estrogens as well as alkylphenols have been detected in municipal effluents (Bennie et al. 1998; Bennie 1999; Lee et al. 2000; Ternes et al. 2001) at concentrations that are similar to those which are known to cause endocrine responses in fish (Jobling and Sumpter 1993; Jobling et al. 1996).

Runoff from Agricultural Activities

Many pesticides currently used in Canada, including mancozeb, endosulfan, cypermethrin, metribuzin and metiram, are suspected EDS (Soto et al. 1995; Teather et al. 2001). In addition, nonylphenol ethoxylates are a component of many commercial pesticide formulations and their degradation products (e.g., nonylphenol) can function as EDS (Jobling and Sumpter 1993). There has been recent concern over the potential for agricultural runoff to affect endocrine function in aquatic species (Environment Canada 1997). Sediments collected from waterways draining areas intensively utilized for potato production in PEI have shown the potential to affect Japanese medaka (*Oryzias latipes*) egg survival, development and time-to-hatch (Gray et al. 2000). At this time it is not known if these effects are specifically related to endocrine disruption or agrochemical residues in sediments.

Relatively high levels of natural estrogens in poultry wastes are capable of entering adjacent streams during field applications (Shore et al. 1993a,b 1995; Nichols et al. 1997) at concentrations that would be expected to cause endocrine responses in fish (Jobling and Sumpter 1993). An examination of runoff after rainfall events suggests that estrogens may be relatively persistent in soils (Nicholls et al. 1997). Hog manure can also contain very high concentrations of natural estrogens (17β-estradiol and its metabolite estrone) compared to municipal effluents (Servos et al. 1998a,b). It was determined that these estrogens can reach streams through tile drainage during field applications and subsequent rainfall. In three manures examined from southwestern Ontario, the concentrations of 17β-estradiol ranged from 1,000 to 27,000 ng/L, several orders of magnitude higher than those measured in final municipal sewage effluents. Equol, a metabolite of phytoestrogens, has recently been identified as an additional estrogenic component of hog manures (Burnison et al. 2000). Normally, manures do not enter surface waters directly but are applied to agricultural fields and enter streams through tile drains or seepage; aquatic exposures to the estrogenic substances contained within manure have not yet been defined.

Municipal treatment plant (sewage) sludge applications to agricultural land are a common practice in Canada. Sludges have been found to contain relatively high concentrations of APEs and their metabolites, including nonylphenol and octylphenol (Servos et al. 1999; Lee et al. 2000). Alkylphenols may be rapidly removed from soils after land application but a significant residual remains in the soil after the application (Servos et al. 1999). Sludges are also expected to contain a variety of other EDS, including natural and synthetic estrogens, derived from partitioning within the sewage treatment system. Unlike final sewage effluents, sludges are expected to contain a wider variety and higher concentration of more hydrophobic chemicals (e.g., nonylphenol). The composition of EDS in sewage sludges may therefore differ considerably from that of final sewage effluents. The potential movement of these compounds from fields has not yet been established.

Contaminated Sediments

Sediments historically contaminated with persistent chemicals linked to endocrin disruption such as PCDD/DFs, PCBs, organochlorine pesticides and heavy metals are associated with several of the Great Lakes' Areas of Concern and other basins affected by industrial activities such as the Fraser River basin (Macdonald et al. 1998). In these areas the concentrations of many chemicals are sufficiently high to produce acutely lethal effects. Present day inputs are significantly reduced relative to historical discharges, and slow declines in sediment concentrations have been observed in the last decade (Department of Fisheries and Oceans 1998; Macdonald et al. 1998). While high levels of POPs and PBTs have been detected in sediments collected from these sites, contamination is generally localized. Many of these chemicals are of sufficient hydrophobicity that they are not readily bioavailable to most aquatic species; however exposures continue through food web accumulations and through resuspension events such as dredging. Resuspension events, localized currents and particulate deposition rates primarily influence sediment concentrations. Limited work on effects associated with contaminated sediments from Hamilton Harbour has shown that responses can be induced in laboratory exposures and with sediment extracts (Munkittrick et al. 1995) but the contributions of known EDS has not been investigated.

Conclusions

- Wide ranges of chemicals that are present in Canadian aquatic environments have been shown to individually have the potential to modulate endocrine function under laboratory conditions. The diversity of chemicals includes almost all classes of environmental contaminants.

- The relationship between dose and response for many EDS is not well defined (especially for non-mammalian endpoints), making it difficult to assess their risk.
- The environmental exposure to many identified EDS is not well characterized and requires additional monitoring.
- Several EDS have the potential to persist and bioaccumulate in food chains. Studies have demonstrated contaminant burdens of these compounds in aquatic biota but specific cause and effect linkages have been difficult to establish.
- The identities, sources and distribution of the specific chemicals responsible for endocrine related responses in mixtures and effluents are unknown. The interactions and bioavailability of these chemicals in complex mixtures is poorly understood.

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