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Assessment of the Effects of Endocrine Disrupting Substances in the Canadian Environment

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Endocrine disruptors are a complex issue that continues to evolve. From a government perspective, the issue of endocrine disruptors is complicated by the inclusion of several related issues, making it difficult to deal with in an effective manner. The sub-issues probably need to be dealt with through different regulatory mechanisms. The endocrine disruptor issues can be divided into three main categories: a) issues associated with subtle responses to compounds that are persistent, lipophilic and capable of biomagnification; b) issues associated primarily with non-persistent and relatively hydrophilic substances in industrial and municipal effluents; and c) issues associated with screening existing and new chemicals for their capability of interacting with the endocrine system in an adverse manner. This paper discusses options for dealing with chemicals found in complex mixtures such as pulp mill effluents, sewage effluents and in-use agricultural chemicals. When studies documented potential concerns about the potential for pulp mill effluents to cause reproductive and endocrine changes in fish, the Government of Canada developed an Environmental Effects Monitoring program as part of the new regulatory package. The EEM program is designed to provide information on whether effects are present in the environment when industry complies with their regulated discharge requirements. Endocrine disruptors have the potential to cause environmental effects with other regulated effluents, and an EEM-type of approach would be capable of identifying situations where effects are present and need to be dealt with.

Key words: **endocrine disruption, environmental effects monitoring, effluents, non-point stressors**

Introduction

Although the "endocrine disruptor" issue was precipitated in the public arena by the publication of *Our Stolen Future* (Colborn et al. 1996), the ability of anthropogenic chemicals to interact with hormone systems and cause subtle biological responses has been known for decades (e.g.,

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NAS 2000). The list of chemicals perceived to be capable of disrupting the endocrine system continues to grow, and the growth will continue as new data are developed. The biological activity associated with exposure to all chemicals is associated with changes to the endocrine system, some in response to the action of the chemical compounds at the target site, and some as secondary responses to the chemical's reactions. Furthermore, the endocrine system is responsible for mediating the animal's responses to all external cues, including changes in diet, photoperiod and behaviour. It can be difficult to distinguish situations where foreign compounds are directly altering the ability of the endocrine system to control physiological processes from situations where the endocrine system of the animal is responding to habitat changes or physiological changes that are indirectly related to the presence of chemicals.

The response of different countries and agencies to the endocrine disruptor issue will depend, in part, on the regulatory environment under which they operate, as well as the regional-specific issues that are driving the issues surrounding endocrine disruptors. For example, the issue is perceived in the U.S. to be predominantly related to an issue of screening effects, whereas in the U.K. it has been perceived as an estrogenic issue (i.e.. SDC 1998) and in parts of Scandinavia as an issue related to human reproductive health and sperm quality (e.g., Sharpe and Skakkebaek 1993). The following is proposed as a discussion paper to facilitate discussion of the potential avenues that can be considered for regulating endocrine disrupting substances (EDSs). It is not meant to be an exhaustive review, but rather an exploration of the existing avenues that can be used within Canada for addressing the issues of EDSs in the environment.

What Issues Are Driving Perceptions About EDSs?

It is important to review the issues and concerns that are driving the endocrine issue. A recent European Union report (Anon. 1999) recognizes a number of concerns, including:

- the consequences of exposure to tributyltin (TBT) and the appearance of imposex in snails;
- the relationships between DDE and egg-thinning in birds;
- the appearance of ovotestis conditions in fish near water treatment plants and coastal areas;
- immune and reproductive impacts in Baltic seals correlated with concentrations of PCBs;
- reproductive concerns surrounding Florida alligators and their potential links with pesticides from the DDT-complex.

The main risk factors noted in the document are the diet for mammals and free-living embryonic stages for aquatic organisms. The list serves to point out that although the list of suspected EDSs has grown substantially in the last 5 years, all of the listed concerns were known at least 5 years ago (and some longer ago than that). The TBT concerns date back to before 1990 (Bright and Ellis 1990), the health of birds back to the

1970s (Fox 1994), estrogenic effects of sewage treatment plants to the early 1990s (Purdom et al. 1994), seals since the 1980s (Reijinders 1986), and potential impacts on alligators to the early 1990s (Guillette et al. 1994).

What Are the Canadian Regulatory Issues Associated with EDSs?

There are numerous science issues that are associated with endocrine disruptors, including the possibility that there are critical developmental stages where organisms may show heightened sensitivity, as well as the possibility of low-dose effects and the presence of yet undefined thresholds for activity. However, from a regulatory viewpoint, there are a number of different issues. It will be difficult to regulate endocrine disruptors on the basis of their ability to interact with endocrine systems because all substances interact directly or indirectly with the endocrine system.

It is important to address how the issue is perceived and to define how the issue should be dealt with in a regulatory sense. The endocrine disruptor issue has been perceived by many to be predominantly an estrogenic issue; others have defined it as the potential for foreign chemicals to interact with the estrogenic, androgenic and thyroidogenic receptors to broaden the issue. Focusing on the physiological mechanism of binding of EDSs to receptors or to physiological responses does not specifically deal with the issue of policy needs. It is necessary to define the issue in terms of the regulatory tools and mechanisms that can be focused to deal with the issue.

For the purposes of examining regulatory requirements, the endocrine issue can be divided into four main components:

a) The need to screen chemicals currently seeking registration for use.
b) The need to screen chemicals that are currently registered for use, including the needs to prioritize which chemicals need to be reexamined, in what order, and for what responses.
c) The need to deal with the consequences associated with compounds that are presently in the environment, and are persistent, biomagnify and are known to be toxic (PBTs), including polychlorinated dioxins and furans (PCDD/PCDFs), PCBs and DDT.
d) The need to develop regulatory tools to deal with compounds interacting with the endocrine system that are not as persistent, are not known to biomagnify and may not be toxic as defined by existing, commonly used toxicity tests (non-persistent, non-biomagnifying and non-toxic [nPnBnTs]). This grouping includes compounds interacting with the endocrine system that are found in pulp mill effluents, sewage effluents, textile effluents, and modern pesticides, etc.

There are a variety of international working groups (i.e., EDSP 2000; OECD via *http://www.oecd.org/ehs/endocrin.htm*) addressing the issues related to screening existing chemicals and chemicals proposed for registration, and they will not be dealt with further here other than to say that Canadian scientists need to continue to play a role in the scientific discussions surrounding the development of policies for regulating these compounds. Regulatory mechanisms exist to regulate the use of these

compounds once effective screening protocols exist, and the responses of concern can be defined and quantified.

A greater concern exists for the latter two categories of compounds. Compounds that are persistent, biomagnify and are known to be toxic (PBTs) include dioxins, PCBs and DDT (among others) that have been, and continue to be, associated with a variety of endocrine responses. New concerns associated with the PBT compounds include concerns that impacts may be occurring at much lower doses than previously thought; there may be inverted v-shaped dose-response curves, there may be low-dose effects, questions related to potential thresholds and potential windows of sensitivity, and possible interactions between chemicals. All of these are key research issues, but from a regulatory perspective, the government knows that it does not want persistent, lipophilic compounds released into the environment. It is possible that no new regulations are needed, although there are concerns and issues with respect to aboriginal/native diets in polar regions. In terms of potential impacts on wildlife, it may be necessary to redefine no-effect levels and what zero discharge means, but it is doubtful that new regulations need to be developed to deal with these compounds, since many of them are already banned. The largest concern from a government perspective should be the last category: compounds that are not persistent and do not biomagnify but have been associated with endocrine responses.

Compounds That Are Not Persistent and Do Not Biomagnify

There have been concerns that compounds in sewage and textile effluents are capable of impacting fish (Jobling et al. 1996). Possible responsible compounds include nonylphenol polyethoxylates (NPEs), natural human estrogens, and estrogens derived from birth control pill metabolites (Mathiesson 1998). These concerns may be independent from those potentially associated with the PBT issue. The compounds are likely not expected to biomagnify, and most of the exposure concerns are related to organisms that are directly exposed in the aquatic environment.

Canadian and Scandinavian studies have shown that effluents from some pulp mills are capable of disrupting the control of sex steroid hormones and pituitary hormone production and circulatory levels, and can be associated with delayed sexual maturity, reduced gonadal size and egg production, and alter secondary sex characteristics (reviewed in Munkittrick et al. 1997, 1998). The physiological responses of fish to pulp mill effluent are not the same as to estrogens (Van Der Kraak et al. 1998). While the identity of the responsible compounds is unclear, it is clear that the responses can be associated with compounds that are water soluble, and that effects can be reversible and short-lived. Studies demonstrated that changes in fish could be detected with some effluents when concentrations approached 1% in the receiving environment, in response to effluents that did not cause acute lethality in rainbow trout at 100% effluent concentrations, and were not associated with sublethal toxicity responses

(Robinson et al. 1994). At some sites, this means that changes were detectable close to 100 km below discharges (Hodson et al. 1992). These responses were seen in wild fish, despite the facts that the effluents were not toxic in traditional toxicity tests, and the responsible chemicals are not persistent and do not appear to biomagnify.

These chemicals or mixtures are associated with impacts on maturity, secondary sex characteristics, gonadal sizes, or with responses in internationally recognized EDS endpoints such as vitellogenin or intersex. The characteristics of these responses are that they are subtle, previously undetected responses in environments that were not surveyed, and are found at concentrations below those thought to be a risk. Chemicals in these effluents that are responsible for altering the endocrine system can cause responses at very low exposure levels, but these environmental responses are not predicted by examination of the effluents' persistence, lipophilicity or acute/chronic toxicity tests. These effluents do not cause any of the responses that would trigger the existing regulatory process, but environmental concerns exist.

There are a number of issues that need to be addressed to deal with chemicals that are non-persistent, non-biomagnifying and are not toxic under existing regulatory tests (nPnBnT). These include:

a) An understanding of the ecological relevance of physiological responses associated with these effluents and mixtures. There are documented responses in terms of potential reproductive endpoints, including vitellogenin induction and alteration of steroid hormones that we do not understand the whole organism or ecological relevance of.
b) An understanding of the significance of whole organism responses such as intersex, both to the performance of the individual, as well as to the population.
c) The development of short-term toxicity tests that can predict the potential of effluents or mixtures to cause endocrine concerns.

The endocrine issue is placing a large priority on chemicals that act through an endocrine mechanism. A critical question for dealing with the nPnBnT issue is whether it is important for the chemicals to be acting through an endocrine mechanism to be a concern. The focus of perceptions on the potential molecular interactions with receptors and on laboratory testing obscures the need to deal with the real issues that developed the EDS issue in the first place. Most international workshops have agreed that *in vivo* whole organism responses will drive the issues, other than for screening. Changes in maturity and gonadal size can occur for reasons other than direct impacts of foreign chemicals on endocrine systems (Munkittrick and McMaster 2000). Some of the stressors in these categories can impact food availability and other ecological responses that can impact reproductive performance (Munkittrick et al. 2000). A focus on EDSs means that needless arguments develop about whether an impact on reproduction (or growth, or development) acts through an endocrine-mediated disruption, when the policy and regulatory need is to deal with

the impact on reproduction (or growth, or development). The question, in terms of environmental responses is, do we want to regulate EDSs or do we want to deal with the issues?

It is important to broaden the thinking surrounding EDSs to be able to deal with situations where we may have previously undetected impacts in situations that we assume current regulations are adequate to protect the environment. It is important to recognize the endpoints where we have concerns, examine receiving environments for these issues and to screen effluents and mixtures for their potential to cause these changes. In terms of potential regulatory mechanisms to deal with nPnBnTs, it would be useful to review how the Canadian government handled the observations of the potential impacts of pulp mill effluents on gonadal size, maturity and reproductive steroid hormones. A similar program has also been developed for metal mines, and reflects the need to detect important changes in growth, reproduction and survival, regardless of whether they are acting through an endocrine mechanism or not.

Environmental Effects Monitoring

A new pulp and paper regulatory package was developed in the early 1990s that included requirements that effluents do not contain detectable levels of dioxins and furans, and new limits on biological oxygen demand, suspended solids and acute toxicity (PPER 1992). In response to concerns about potential ongoing impacts near effluent discharges, an Environmental Effects Monitoring (EEM) program was included in the regulatory package. The Canadian program represents an industry-funded cyclical survey to determine whether impacts exist near their discharges when they are in compliance with existing regulations. While a few other countries have developed EEM programs for pulp and paper mills, they are either utilized for new developments (Australia; Keough and Mapstone 1995, 1997; Mapstone 1995; Terrens et al. 1998) or tools to be considered during the development of water licenses (Sweden: Thoresson 1993; Swedish EPA 1997). An EEM program has also just completed development for the metal mining industry and represents a consensus program developed with the cooperation of industry, government and other stakeholders (*http://www.ec.gc.ca/eem/english/METAL.HTML*), and includes additional site-specificity, flexibility and monitoring options. The pulp and paper program defines the basic monitoring program, whereas the metal mining program is focused on key questions that industry must address, and default options for monitoring to be considered.

To meet the objective of the EEM program, facilities are required to monitor fish populations as an indicator of effect on fish, monitor benthic invertebrate communities as an indicator of effects on fish habitat, and conduct fish tissue or tainting analysis as an indicator of effects on the use of fisheries resources. Within the EEM program, an effect is defined as a statistical difference in measurements taken for these indicators between sites exposed to effluent and reference sites of similar characteristics that are not exposed to effluent (Requirements Documents, Environment

Canada 2000). Once an effect is identified, the EEM program requires the regulated industry to determine the magnitude and geographic extent.

The fisheries component of the pulp and paper EEM requires that facilities collect information on the size, growth, organ size and reproductive investment of the adults of two fish species. It assumes that sites that are unable to find impacts on the age, growth and reproductive investments are not impacting the fish in the near-field receiving environment (effluent concentration >1%). After the completion of the first cycle of EEM for pulp and paper in April of 1996, the program was significantly redesigned (Environment Canada 1997). The first cycle was limited by the decision that all mills would have to undertake an identical program. The redesign of the program included the construction of a series of decision trees that enabled some better site-specificity. Pulp and paper EEM programs monitor on a 3-year cycle, and cycle 2 reported on April 1, 2000; those data are currently under review.

What is the Potential Role of EEM for Helping To Deal with the EDS Issue?

The EEM program was developed, in part, because of the concerns that pulp mill effluents were associated with changes in terms of maturity and reproduction of fish. It is possible to apply EEM to other situations, and a potential mechanism is to solicit the participation of provinces to require EEM-type studies under their existing certificates of approvals and discharge permits. The priority is to understand where we have issues. It is possible to use existing regulatory tools when we understand the issues, but we do need new chronic toxicity tests developed to predict the potential of effluents and chemicals to be associated with environmental responses of concern.

A potential regulatory approach to deal with nPnBnT-type responses is to identify the specific concern in priority situations, recognizing that in some cases the concern may not be related to chemicals that act as endocrine disruptors. If the problems associated with changes in whole organism performance are habitat-related, it should be possible to use the habitat protection provisions of the *Fisheries Act*. If the issues are contaminant-related, there are existing aspects of the *Fisheries Act* or CEPA that can be used. There are clear research needs, including:

a) where do we have concerns?
b) what is the relevance of internationally recognized endocrine endpoints?
c) how can we detect new issues or recognize the potential of emerging issues?

Conclusions

There is a concern that low-level and/or multigenerational effects are occurring, and a concern that there is not a mechanism to identify these issues. Some of these may not act through an endocrine disruption mech-

anism. The conclusion is that the concern should really be focused on sublethal effects on growth, reproduction and developmental endpoints rather than on a specific mode or mechanism like endocrine disruption. Tests do exist that can detect many of the endpoints of concern (e.g., fathead minnow life cycle bioassay), but these tests are not routinely used due to complexity and expense.

The EDS issue can be dealt with effectively under current approaches if it is divided into issues associated with a) regulating new and existing chemicals for use; b) dealing with concerns about in-place contaminants that are persistent, biomagnifying and toxic (PBTs); and c) concerns associated with the discharge or use of chemicals that are not persistent, do not magnify and are not toxic (nPnBnT) in existing regulatory tests. The latter category could use an approach like Environmental Effects Monitoring to identify situations that are associated with whole organism changes. Environmental monitoring programs require additional resources, and need to be designed to identify new issues and complement mechanistic and toxicology studies.

Such an approach would benefit from a monitoring and assessment framework that encompasses the needs for impact assessment, environmental effects monitoring, cumulative effects assessment and regional monitoring, and allows the information to be cross-referenced and utilized for a variety of purposes.

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