

## Endocrine Disruption: Why Is It So Complicated?

ANDREA L. LISTER AND GLEN J. VAN DER KRAAK\*

*Department of Zoology, University of Guelph, Guelph, Ontario N1G 2W1*

---

The intricate nature of the vertebrate endocrine system creates several challenges that impede the understanding of the threats that endocrine disrupting substances (EDS) may pose to both humans and wildlife. While there are many similarities in the organization of endocrine communication across vertebrate classes, there are differences in hormonal activities and regulated events which makes generalizing EDS effects across species difficult. Aspects of endocrine functioning that may be affected by EDS include the biosynthesis, transport or availability, and metabolism of hormones. Also, EDS may interact with hormone receptors, which is a feature exploited by researchers as a screening method to identify potential EDS. There are many factors to consider in regards to the effects of potential EDS on endocrine functioning, including the timing of exposure, species-specific differences, and a multitude of other factors, which may impinge directly on the physiological endpoints used to determine if endocrine disruption has occurred. It is important to understand the status of the endocrine system before attempting to interpret reproductive status or the general health of the population. This paper provides an overview of the endocrine physiology of vertebrates and a description of the mechanisms by which EDS may affect endocrine function. As well, some of the factors that complicate our understanding of the relationship between exposure to EDS and compromised health in different vertebrate species are included.

*Key words:* endocrinology, hormone biosynthesis, hormonal actions, endocrine disruptors, endocrine toxicology

---

### Introduction

Over the last decade, there has been great interest in both the scientific community and the general public in regards to possible alterations in the functioning of the endocrine systems of humans and wildlife as a result of chemical exposure. The concept that environmental chemicals influence aspects of growth, reproduction and development in vertebrates is not new and has gained renewed attention since the publication of the results of a work session in 1991 entitled “Chemically Induced Alterations in Sexual Development: The Wildlife/Human Connection” (Colborn and Clement 1992) and the book “Our Stolen Future” (Colborn et al. 1996). Studies summarized in these and other publications have highlighted numerous examples of compromised growth and reproduc-

---

\* Corresponding author; gvanderk@uoguelph.ca

tion, altered development and abnormal behaviour in various taxa including invertebrates, fish, amphibians, reptiles, birds and mammals. These identified effects can be correlated or in some cases causally linked with exposure to endocrine disrupting substances (EDS) (Rolland et al. 1997; Ankley et al. 1998; Kendall et al. 1998; Van Der Kraak 1998; National Research Council 1999).

At a recent meeting held by the Organization of Economic and Cooperative Development and the International Program on Chemical Safety, an endocrine disrupting chemical has been defined as an exogenous substance or mixture that alters the function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny or (sub)populations. There are numerous chemicals in the environment that are hormonally active and these encompass a variety of chemical classes, including natural and synthetic hormones, plant constituents, pesticides, compounds used in the plastics industry and in consumer products, as well as industrial by-products and effluents. These chemicals are often pervasive and persistent and, as a result of extensive use or global transport, have a ubiquitous distribution on the earth, whereas the identification of other endocrine-active chemicals may be difficult due to their short half-lives or instability in the environment. This broad range of chemical characteristics makes it difficult to establish exposure/response relationships, which compounds the uncertainties when conducting human and ecological health assessments.

Defining the extent to which environmental chemicals affect the functioning of the endocrine system and thereby contribute to adverse health effects in wildlife and humans is a complex issue. Exposure to chemicals that affect the functioning of the endocrine system does not necessarily result in an adverse outcome since the response depends on the level, duration, and timing of exposure. It is understanding the linkage between exposure and outcome that poses the greatest challenge to researchers. Another major challenge relates directly to the remarkable complexity of the endocrine system. We now realize that multiple hormones, which control complicated events like reproduction and development, work through vastly different biochemical mechanisms to produce the same effect on the individual. Individual hormones may cause different effects depending on the nutritional status, age, and gender of the individual or the cell or tissue type in which it acts. Furthermore, hormones and EDS may exert their effects during critical periods of development but these effects may not be manifested until much later in the individual's lifetime. Predicting the responses induced by potential EDS across different vertebrate classes is difficult because of subtle differences in the organization of endocrine communication, the nature of the receptors and signaling pathways, as well as the presence of different developmental events controlled by hormones (e.g., smoltification in salmonids, metamorphosis in amphibians). Numerous similarities also exist in the functioning of components of the endocrine system irrespective of the species. These similarities have aided in our understanding of the mechanisms and effects of potential EDS.

The purpose of this paper is to provide an introduction to some of the issues and uncertainties that influence our understanding of the risks posed by EDS in wildlife and humans. Specifically, this paper provides 1) a brief overview of endocrine physiology in vertebrates, 2) a description of the mechanisms by which EDS may affect endocrine function, and 3) an introduction to some of the factors that complicate our understanding of the relationship between exposure to EDS and compromised health.

### Endocrine Communication

Communication within the vertebrate endocrine system is facilitated by hormones, which are the chemical messengers that regulate and coordinate diverse physiological processes, including reproduction, growth, maintenance of the internal environment and energy availability (Griffin and Ojeda 1992; Hadley 1996; Norris 1998). Although there are a few notable exceptions (i.e. retinoids), hormones fall into four main categories: 1) amino acid derivatives, 2) proteins, 3) steroids, and 4) eicosanoids. The first of these are hormones derived from single amino acids and include the catecholamines, epinephrine and dopamine. The thyroid hormones, thyroxine (T<sub>4</sub>) and triiodothyroxine (T<sub>3</sub>), are iodinated derivatives of tyrosine. The protein hormones range in size from the three amino acid thyrotropin-releasing hormones, to long chain proteins (about 200 amino acids in the case of growth hormone) and complex dimeric glycoproteins such as follicle stimulating hormone (FSH) and luteinizing hormone (LH) with molecular weights in excess of 30 kDa. The steroid hormones are derivatives of cholesterol. These can be grouped into two types: those with an intact steroid nucleus such as the gonadal (androgens, estrogens and progestins) and adrenal (glucocorticoid and mineralocorticoid) steroids and those with a broken steroid nucleus such as vitamin D and its derivatives. Finally, the eicosanoids are long chain polyunsaturated fatty acid derivatives, which include the prostaglandins, thromboxanes and leukotrienes.

There is considerable homology in the structure of many hormones across the vertebrate classes (Norris 1998; Van Der Kraak et al. 1998a). Certain hormones, such as the catecholamines and thyroid hormones, are the same from fish to humans. Although the steroid hormones are synthesized from common precursors in all vertebrates, there may be differences between species in the predominant metabolites. For example, in fish 11-oxygenated androgens, especially 11-ketotestosterone are present in high quantities in the blood and may represent the biologically active androgen responsible for stimulating secondary sexual characteristics, reproductive behaviour and spermatogenesis (Borg 1994), while in mammals, testosterone and 5 $\alpha$ -dihydrotestosterone are largely responsible for these events.

In addition to the steroids, the structure of protein hormones and their genes can be highly conserved in different vertebrates. For example, gonadotropin-releasing hormone (GnRH), which is well known for its action in releasing gonadotropins from the pituitary of vertebrates, is present in all species studied to date, as well as in some invertebrates. So far, 13 different forms of GnRH have been identified by primary structure or

**Table 1.** Comparison of gonadotropin-releasing hormone sequences in different animals (modified from Carolsfeld et al. 2000)

GnRH	Positions of amino acids																					
	1	2	3	4	5	6	7	8	9	10												
Herring	p	Glu	-	His	-	Trp	-	Ser	-	His	-	Gly	-	Leu	-	Ser	-	Pro	-	Gly	-	NH <sub>2</sub>
Catfish	p	Glu	-	His	-	Trp	-	Ser	-	His	-	Gly	-	Leu	-	Asn	-	Pro	-	Gly	-	NH <sub>2</sub>
Seabream	p	Glu	-	His	-	Trp	-	Ser	-	Tyr	-	Gly	-	Leu	-	Ser	-	Pro	-	Gly	-	NH <sub>2</sub>
Chicken-II	p	Glu	-	His	-	Trp	-	Ser	-	His	-	Gly	-	Trp	-	Tyr	-	Pro	-	Gly	-	NH <sub>2</sub>
Dogfish	p	Glu	-	His	-	Trp	-	Ser	-	His	-	Gly	-	Trp	-	Leu	-	Pro	-	Gly	-	NH <sub>2</sub>
Chicken-I	p	Glu	-	His	-	Trp	-	Ser	-	Tyr	-	Gly	-	Leu	-	Gln	-	Pro	-	Gly	-	NH <sub>2</sub>
Mammal	p	Glu	-	His	-	Trp	-	Ser	-	Tyr	-	Gly	-	Leu	-	Arg	-	Pro	-	Gly	-	NH <sub>2</sub>
Salmon	p	Glu	-	His	-	Trp	-	Ser	-	Tyr	-	Gly	-	Trp	-	Leu	-	Pro	-	Gly	-	NH <sub>2</sub>
Lamprey-III	p	Glu	-	His	-	Trp	-	Ser	-	His	-	Asp	-	Trp	-	Lys	-	Pro	-	Gly	-	NH <sub>2</sub>
Guinea pig	p	Glu	-	Tyr	-	Trp	-	Ser	-	Tyr	-	Gly	-	Val	-	Arg	-	Pro	-	Gly	-	NH <sub>2</sub>
Tunicate-I	p	Glu	-	His	-	Trp	-	Ser	-	Asp	-	Tyr	-	Phe	-	Lys	-	Pro	-	Gly	-	NH <sub>2</sub>
Tunicate-II	p	Glu	-	His	-	Trp	-	Ser	-	Leu	-	Cys	-	His	-	Ala	-	Pro	-	Gly	-	NH <sub>2</sub>
Lamprey-I	p	Glu	-	His	-	Tyr	-	Ser	-	Leu	-	Glu	-	Trp	-	Lys	-	Pro	-	Gly	-	NH <sub>2</sub>

complementary DNA analysis and each has a length of 10 amino acids with identical residues in positions 1, 4, 9 and 10 (Table 1; Carolsfeld et al. 2000). In the case of protein hormones, amino acid similarities of 40 to 70% (growth hormone, luteinizing hormone, follicle stimulating hormone) and higher (insulin-like growth factor 1) between fish and mammals are common (reviewed in Van Der Kraak et al. 1998a). There are few examples of unique hormones in vertebrates, despite some very different life history strategies that are under hormonal regulation. One exception is somatolactin, a hormone that has been identified in the pituitary gland of fish, which affects a variety of physiological processes, including growth, reproduction and osmoregulation (Rand-Weaver et al. 1991; Ono and Kawauchi 1994). While somatolactin has no apparent homolog in higher vertebrates, it does bind to growth hormone receptors in mammals.

The unifying feature of hormone action is the presence of receptors on target cells, which bind a specific hormone with high affinity and stereospecificity, are responsible for the transmission of a hormone signal into a biological response. Hormones that cannot readily diffuse into cells (peptides, proteins, amino acid derivatives, eicosanoids) exert their effects primarily through interaction with membrane bound receptors. In these cases, binding of a hormone to its membrane-bound receptor results in the initiation of intracellular signaling pathway(s) that lead to the generation of second messengers such as cAMP, inositol trisphosphate or diacylglycerol or changes in flux of ions (e.g., calcium), which trigger intracellular events (Van Der Kraak and Wade 1994). Another mechanism of

hormone action is shown by steroid hormones, thyroid hormones and retinoic acid, which exert their effects by binding to nuclear receptors that function as ligand-dependent transcription factors. Their hormonal effects are manifest through interaction with DNA and changes in the expression of a number of genes.

The intracellular signaling pathways and processes occurring downstream of hormone-receptor binding are conserved well in vertebrates. The signal transduction pathways initiated downstream of catecholamine and peptide hormone binding (e.g., interaction with heterotrimeric G-proteins, adenylyl cyclase, tyrosine kinase activity) appear to function in a similar manner across vertebrates (Chang and Jobin 1994; Van Der Kraak and Wade 1994). Similarly, the genomic structure of upstream regulatory portions of the steroid responsive genes are highly conserved across vertebrates (Van Der Kraak et al.1998a). However, like the protein hormones, there are subtle differences in the amino acid composition of hormone receptors across species. This has direct bearing on a hormone receptor’s binding affinity for both endogenous hormones and environmental chemicals.

Perhaps the most unique feature of endocrinology in vertebrates is the diverse nature of the processes that are under hormonal control. Placentation, lactation, vitellogenesis, tolerance to different osmotic environments, and metamorphosis represent but a few of the complex events that are hormonally regulated in vertebrates. When considering the topic of hormones and evolution, a pioneer of comparative endocrinology wrote, “It is not the hormones that evolve, but the uses to which they are put.” (Gorbman et al. 1982). Understanding the diverse roles of hormones in the physiology of vertebrates is a challenge. The intricate nature of the endocrine system makes it difficult to understand how potential EDS may modulate certain aspects of endocrine functioning.

The actions of hormones fall into two major categories (Table 2). First, hormones can be organizational whereby their actions occur during critical periods of development and induce permanent effects. The organizational effect of hormones is demonstrated by the actions of sex steroids, which permanently alter the development of the brain, reproductive tract and the

**Table 2.** A summary of cellular responses mediated by hormones acting through organizational or activational mechanisms

Cellular responses	
Organizational	Activational
Early in development	Throughout development
Irreversible	Reversible
Permanent	Transitory (homeostatic)

accessory sexual organs. This is an important consideration with respect to environmental chemicals as they could transcend the actions of endogenous hormones and have a permanent effect on physiological processes. In contrast, hormones can be activational as their actions cause transient changes in a myriad of cellular processes that occur in target organs in response to hormone action. The rapid effects of glucagon and insulin on glucose homeostasis typifies the activational effects of hormones.

The regulatory events controlling hormone secretion involve similar processes irrespective of the vertebrate species. The secretion of a hormone is subjected typically to a negative feedback loop whereby an elevation in the hormones levels triggers a negative biological response, which counteracts its secretion. This loop functions to maintain hormone levels within a homeostatic range. A common negative feedback mechanism involves testosterone. In this case, high levels of testosterone secreted from the testis negatively influence the secretion of the trophic hormones, luteinizing hormone-releasing hormone and luteinizing hormone from the hypothalamus and pituitary, respectively. An emerging issue involving the regulation of hormone release is the realization that there are multiple and interacting factors that govern the release of hormones within the pituitary and peripheral endocrine tissues. This complexity is demonstrated by the existence of greater than 15 hormones and local growth factors which control the release of luteinizing hormone in fish and mammals (Van Der Kraak et al. 1998b). This multiplicity of endocrine regulation is not characterized particularly well in lower vertebrates and there are differences in the relative importance of the regulators between species. This will create an additional challenge when evaluating the possible effects of EDS on hormone secretion in different species.

### **Mechanisms of Endocrine Disruption**

It is a complex task not only to identify the compounds in the environment responsible for endocrine disruption, but also to discover the mechanisms by which they disrupt endocrine function. The complexity of the endocrine system creates several different opportunities by which EDS may act to modulate endocrine functioning including aspects of the biosynthesis, transport or availability, and metabolism of endogenous hormones. As well, the activities of hormones may be affected by EDS interacting with hormone receptors and signaling processes.

#### **Alterations in Hormone Biosynthesis**

The biosynthesis of steroid hormones is a multistep process that involves the cleavage of cholesterol to other sterol precursors by P450 monooxygenase enzymes. A variety of enzymatic reactions that occur in the smooth endoplasmic reticulum result in intermediate products that may be hormonally active as well. Environmental compounds that change the availability of precursors such as cholesterol or alter the activity of enzymes within the steroid biosynthetic pathway may alter the syn-

thesis of steroid hormones. Steroid biosynthesis may be affected greatly by contaminants that either induce or inhibit specific monooxygenases that are involved in the production of steroids (Van Der Kraak et al. 1998a). An important example involves aromatase, the enzyme responsible for the conversion of testosterone (and other androgenic metabolites) to estrogens, which is inhibited by a variety of synthetic (e.g., fenarimol) and natural (e.g., flavinoids) compounds that reduce the synthesis of 17 $\beta$ -estradiol (National Research Council 1999). Alterations in the biosynthesis of sex steroid hormones as a result of exposure to environmental compounds have been documented in a variety of wildlife species (McMaster et al. 1996; Fairbrother et al. 1998).

Due to the different processes involved in their synthesis, EDS exert different effects on the biosynthesis of peptide and protein hormones compared with steroid hormones. In addition to potential effects on protein secretion, there are numerous other steps involved in the synthesis of protein hormones that may be affected by EDS. These hormones are synthesized in a manner similar to other secreted proteins where regulation of gene expression and a number of posttranslational modifications (e.g., peptide cleavage, phosphorylation and glycosylation) are involved in the physiological regulation of protein hormone synthesis and secretion. Should these processes be targeted by the actions of EDS, changes in hormone homeostasis may result. For example, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) reduces the bioactivity of corticotropin hormone (ACTH) secreted from the anterior pituitary in rats, possibly by altering the posttranslational modification of this hormone (Bestervelt et al. 1993). In contrast to the considerable focus on EDS modulation of steroid biosynthesis in vertebrates, relatively little is known regarding potential effects of environmental chemicals on protein hormone synthesis and regulation. In either case, it is important to consider whether or not the effect of the contaminant is direct upon the hormonal tissue (e.g., gonad, adrenal gland) or a consequence of changes in endogenous regulators (e.g., luteinizing hormone, ACTH).

### **Alterations in the Transport/Availability and Metabolism of Hormones**

The biological activity of EDS is not necessarily dependent on the amount of a compound in the circulation, but rather the amount of compound available to cells (Guillette et al. 1995). Most endogenous hormones are bound to plasma proteins while a very small amount circulates in a free form. For example, sex steroids (e.g., testosterone, 17 $\beta$ -estradiol) bind to sex hormone binding globulin, glucocorticoids and progesterone bind to corticosteroid-binding globulin, and thyroid hormones and retinoids (vitamin A derivatives) bind to thyroid-retinol binding globulin. Environmental compounds that act as ligands for hormone receptors and are present in relatively higher concentrations in the bloodstream, such as androgenic and estrogenic compounds, may also bind these plasma proteins and displace the endogenous hormones from binding sites. Moreover, some studies have suggested that plasma binding proteins,

such as sex hormone binding globulin, facilitate the transport of sex steroids into hormone-responsive tissues and may do the same with EDS (Rosner 1990; Nakhle and Rosner 1996).

The purpose of hormone metabolism is to form biologically inactive compounds that will be degraded or excreted from the body. The biochemical processes involved (e.g., hydrolysis, conjugation) occur primarily in the liver, kidney, or intestine depending on whether the hormone is a steroid or a protein. Environmental chemicals may affect the metabolism or excretion of hormones by altering the activities of specific enzymes responsible for the degradation of the hormones. Steroids undergo a series of reactions that are catalyzed by P450 monooxygenase enzymes in the liver prior to being conjugated with glucuronide or sulphate and subsequently excreted. Therefore, chemicals that induce or inhibit monooxygenase enzymes, such as TCDD (Goldstein and Safe 1989; Peterson et al. 1997), have the potential to affect the metabolic degradation of hormones (Van Der Kraak et al. 1998a).

### **Alterations in Hormonal Activities**

The realization that environmental chemicals have the capacity to interact with hormone receptors has been a major driving force behind the concept of endocrine disruption. EDS typically bind to the receptor with a much lower affinity and trigger a less pronounced response than the endogenous hormone itself. However, a qualitatively similar response may be generated by the chemical compared with the hormone. While data from ligand binding studies do not predict *in vivo* potencies or whole animal responses to EDS, they do provide a basis from which to investigate the effects of certain compounds in greater detail.

Probably the best characterized mechanism through which some environmental compounds exert their endocrine effects is through interactions with the estrogen receptor (Shelby et al. 1996; Zacharewski 1997; National Research Council 1999). Several other hormone receptors have been found to bind environmental chemical, including the androgen, progestin and retinoic acid receptors, as well as the sex hormone binding globulin (Harmon et al. 1995; Kelce et al. 1995; Peterson et al. 1997; Danzo 1997). Certain chemicals also bind to hormone receptors and do not initiate gene transcription and function as receptor antagonists. In particular, many of the compounds that have been shown to bind to the androgen receptor function as antagonists, including the metabolites of vinclozolin and *p,p*-DDE (Kelce et al. 1994; Peterson et al. 1997). This mechanistic feature of chemicals interacting with hormone receptors, as well as other cellular and molecular measures like gene activation tests, has been instrumental as a screening method for suspected EDS and in defining their mechanism of action.

While it is recognized that the structure of an environmental chemical is a critical determinant in its ability to interact with a hormone receptor, the exact structural requirements are largely unknown and can be met by a diverse range of compounds. Again, the classic example involves the estrogen receptor which has been found to bind numerous compounds,

including natural products, environmental pollutants, pharmaceuticals, and industrial compounds, many of that have been classified as potential EDS (Preziosi 1998; National Research Council 1999).

### Complexities of Endocrine Disruption

Defining the extent to which environmental chemicals affect the functioning of the endocrine system and thereby contribute to adverse health effects in wildlife and humans is a complex issue. The diversity of responses observed in wildlife exposed to environmental chemicals (Van Der Kraak 1998) highlights the inapplicability of using a single approach to investigate endocrine disruption and that the understanding of cause and effect relationships of EDS exposed individuals is complex. A few specific complications in understanding the risks of potential environmental EDS have arisen in recent years and involve 1) the complex mechanisms through which EDS may exert their effects, 2) the difficulties in establishing cause and effect relationships, 3) understanding the linkage between physiological responses and whole organism performance and 4) the relationship between exposure to EDS and the sensitivity of certain developmental life stages.

### Confounding Mechanistic Factors of EDS

Our lack of understanding of the mechanisms of many environmental chemicals, combined with the ability of certain chemicals to affect endocrine functioning through multiple mechanisms, complicates our understanding of the risks they pose to the fitness of wildlife and humans. The pleiotropic nature of certain chemicals has been shown by their ability to bind to several different nuclear hormone receptors. For example, metabolites of vinclozolin and p,p-DDE have been found to bind to the

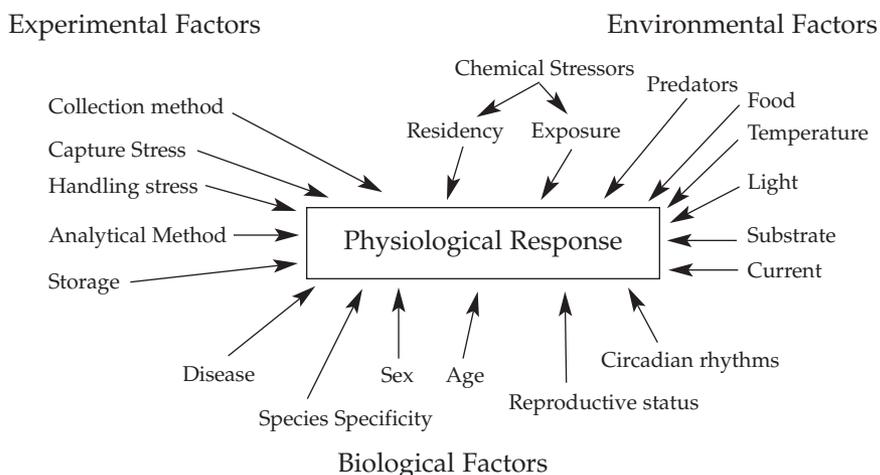


Fig. 1. A summary of various factors influencing physiological endpoints in wildlife.

estrogen, androgen, and progesterone receptors with varying affinities (Kelce et al. 1994; Laws et al. 1996). As well, environmental chemicals may elicit responses through both receptor and non-receptor mechanisms. The phytoestrogen, genestein binds to the estrogen receptor and inhibits tyrosine kinase activity (Akiyama et al. 1987). There are also examples of EDS acting through receptors that are not traditionally viewed as regulators of endocrine physiology. This is shown by the activities of TCDD and related compounds through their broad spectrum of antiestrogenic effects mediated by the aryl hydrocarbon receptor, which is a member of the basic helix-loop-helix receptor superfamily. Male and female rodents exposed to TCDD exhibit a number of endocrine effects, including decreases in secondary sex organ weights, altered sexual behaviour, and delayed onset of puberty (Peterson et al. 1993; Safe 1995).

Another factor that makes predicting the mechanisms of EDS in different vertebrates difficult is that their actions and effects may vary from one species to another. For example, studies using different vertebrates characterized DDT as an environmental estrogen, and DDE as an antiandrogen, whereas salamander larvae responded differently in that DDT had an anti-estrogenic action and p,p-DDE was estrogenic (Clark et al. 1998). As well, species may exhibit significant differences in their hormone receptor binding affinities for environmental chemicals. For example, rainbow trout ER (rtER) has a 10-fold lower binding affinity for 17 $\beta$ -estradiol than the human ER (hER) (Le Drean et al. 1995). The rtER also exhibits a 5-fold lower affinity for DES when compared to its affinity for 17 $\beta$ -estradiol, while DES has a greater affinity for the hER than 17 $\beta$ -estradiol (Petit et al. 1995). These differences in hormone receptor affinities add to the complexities involved with generalizing potential effects across species and establishing the risks that EDS pose.

### **Cause and Effect Relationships**

Defining the association between chemical exposure and physiological dysfunction often involves some degree of reasoned speculation. The presence of a compound with an identified mechanism of action in the environment or in a target tissue offers the possibility that such exposure is responsible for the observed effects. This connection is strengthened when the observed effects are consistent with what is known of the mechanism of action of the chemical and when levels of exposure are relevant to these effects. However, an overriding concern with all ecotoxicological investigations is that a variety of factors, in addition to endocrine disruption, can adversely impact growth, reproduction and survival (Fig. 1). Quite often these are ignored when assessing wildlife populations for the effects of suspected EDS. Food availability, disease state, competition and loss of habitat are significant stressors to wildlife, yet our understanding of how these contribute to physiological fitness is often inadequate (Van Der Kraak 1998; Munkittrick and Van Der Kraak 1999). Often these factors can impinge directly on many of the endocrine measures and physiological endpoints, which are used to evaluate wildlife for the effects of EDS. A range of other

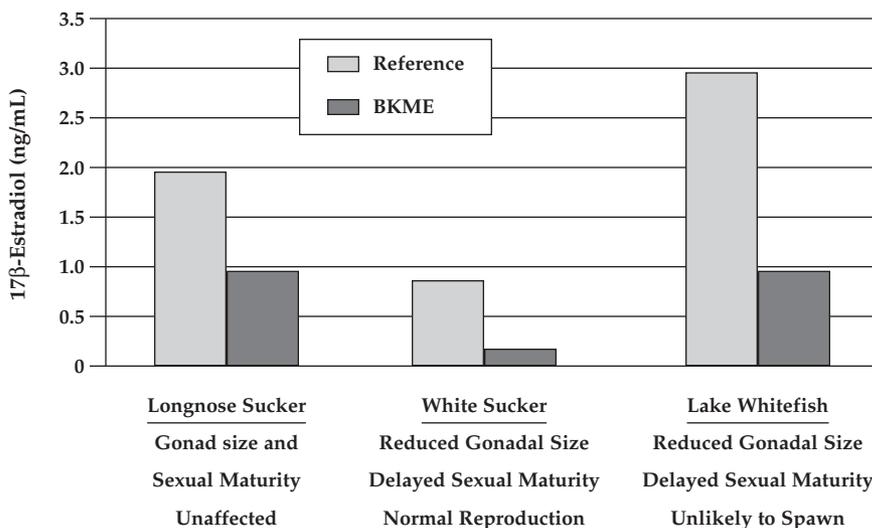


Fig. 2. Relationship between 17β-estradiol titres and reproductive outcomes in three species of fish exposed to bleached kraft mill effluent from the mill at Terrace Bay, Ontario.

factors, including sex, age, season and reproductive state as well as genetics can contribute to variability in these markers. The sampling method and handling and capture stress can also affect physiological measurements and these need to be closely monitored and standardized when sampling wildlife. Interpretation is often complicated in the absence of data on normal ranges for physiological or developmental parameters.

Another feature of wildlife studies is that comparisons are often made in relation to preselected reference locations. Given the range of non-chemical factors, which influence physiological endpoints, there can be questions of whether reference populations are appropriate for comparing responses in exposed populations. Admittedly, this is a concern not only for studies of EDS but for environmental toxicology in general. Wildlife are subjected to multiple stressors and separating out the incremental risks associated with EDS relative to other anthropogenic stressors is a major undertaking (Munkittrick and Van Der Kraak 1999; Van Der Kraak et al. 2001. In the absence of cause and effect information, there is the risk of public and political pressure to eliminate suspected EDS from the environment when they in fact may have little or no effect on physiological performance.

**Linking Physiological Responses to Whole Organism Effects**

Researchers increasingly rely on the use of physiological endpoints (e.g., measurements of circulating hormone levels, histological changes, expression of hormonally regulated proteins) to evaluate the effects of suspected EDS in wildlife and humans. However, there is uncertainty in how these responses may relate to the performance of the individual or

population fitness. This was illustrated in studies evaluating changes in the levels of circulating sex steroids in fish (i.e., longnose sucker, white sucker, lake whitefish) exposed to pulp mill effluent (Van Der Kraak et al. 2001). In this study, the degree of impairment of circulating sex steroid levels was not predictive of reproductive outcomes (Fig. 2). There are many other factors that need to be considered in these types of extrapolations, including the hormone affected, degree of impairment, developmental stage, and the relative species sensitivity. Generally, it is difficult to assess whether or not transient changes in physiology brought on by exposure to chemicals, which affect endocrine processes, have a functional effect on the individual.

Studies by Norris (2000) have brought to the forefront another issue that further complicates the interpretations of physiological endpoints. Norris and coworkers observed that plasma cortisol levels in brown trout living in both clean and metal-contaminated waters were similar. However, histological examination of adrenal cortical tissue of fish from the contaminated site revealed chronic stimulation by ACTH as well as increased numbers of CRH immunoreactive cells. This demonstrates that the metal-exposed fish are hypersecreting ACTH and CRH to maintain base line cortisol levels. Subsequent studies showed that the stress response in fish chronically exposed to metals was impaired relative to controls (Norris, 2000). This example illustrates that endocrine disruption may not be obvious until the individual is challenged regardless of their "normal" physiological appearance. By measuring a static endpoint, like cortisol, the dynamic nature of the stress response mechanism in vertebrates is neglected and potential EDS may go unnoticed without consideration of the organism's response to additional stressors. It is important to understand the status of the endocrine system before attempting to interpret reproductive status, growth, or general health of the population.

### **Sensitive Life-Stages and Species Diversity**

The reorganization of embryos by exposure to EDS during critical periods of embryonic development is of great concern yet few studies have explored this issue. For example, exposure to estrogenic chemicals during a critical period of embryonic development can permanently modify the organization of the reproductive, immune and nervous systems (Guillette et al. 1995). Organizational modifications have been reported for alligators collected from Lake Apopka, Florida, that were exposed to p,p'-DDE during embryonic development periods. The gonads of male and female juvenile alligators are permanently modified by exposure to this EDS. The organizational influences of EDS was brought to the forefront after the effects of diethyl stilbestrol on the developing mammalian embryo became known (National Research Council 1999). It is important to recognize that critical windows of exposure to EDS exist and the responses may not manifest until later in the individual's lifetime.

Our limited knowledge of the basic endocrinology and physiology, particularly during periods of development, creates further challenges in

understanding the risks posed by environmental chemicals. Scientists are also dealing with species which use a diversity of reproductive strategies (e.g., oviparity, ovoviviparity, viviparity, delayed implantation) and often have unique developmental characteristics (e.g., smoltification, metamorphosis), which generally have unknown sensitivities to EDS. Although the endocrine systems across vertebrates are quite similar, the differences that exist in the regulation and actions of hormones makes it difficult to predict or generalize responses of different species to both endogenous hormones and potential EDS.

### Conclusions

The basic design of hormones and endocrine regulation across the vertebrates has been highly conserved and these similarities have aided in our understanding of the mechanisms and effects of potential EDS. The structure of protein hormones, hormone receptors, as well as differences in hormonally regulated events have changed during the course of evolution. Consequently, it is necessary to be cautious in generalizing the mechanisms and possible effects of environmental chemicals in different species. Concerted efforts must be made to link the responses observed at the biochemical or physiological level to the whole organism. Relying solely on changes in endocrine parameters may result in an over estimation of the potential of environmental chemicals to affect the individual. However, understanding the mechanisms by which EDS alter endocrine systems may provide researchers with a clearer view of the potential risks that the contaminants pose at the whole organism level. If more research efforts were directed at understanding the basic endocrinological processes in a variety of species, it may be possible to better understand the risks posed by EDS exposure, particularly during critical periods of development where considerable uncertainty exists. Given the complexity of the endocrine system and the processes under hormonal control, there will continue to be uncertainty in regards to the extent to which EDS are a threat to wildlife and human populations.

### References

- Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, Shibuya M, Fukami Y. 1987. Genestein, a specific inhibitor of tyrosine specific protein kinases. *J. Biol. Chem.* **262**:5592–5595.
- Ankley G et al. 1998. Overview of a workshop on screening methods for detecting potential (anti-) estrogenic/androgenic chemicals in wildlife. 1998. *Environ. Toxicol. Chem.* **17**:68–87.
- Bestervelt LL, Pitt JA, Nolan CJ, Piper WN. 1993. TCDD alters pituitary-adrenal function II: Evidence for decreased bioactivity of ACTH. *Neurotoxicol. Teratol.* **15**:371–376.
- Borg B. 1994. Androgens in teleost fishes. *Comp. Biochem. Physiol.* **109C**:219–245.
- Carolsfeld J, Powell JF, Park M, Fischer WH, Craig AG, Chang JP, Rivier JE, Sherwood NE. 2000. Primary structure and function of three releasing hormones, including a novel form, from an ancient teleost, herring. *Endocrinology* **141**:505–512.

- Chang JP, Jobin RM.** 1994. Regulation of gonadotropin release in vertebrates: a comparison of GnRH mechanisms of action, p. 41–51. *In* . Davey KG, Peter RE, Tobe SS (ed.), *Perspective in comparative endocrinology*. National Research Council of Canada, Ottawa.
- Clark E, Norris DO, Jones R.** 1998. Interactions of gonadal steroids and pesticides (DDT/DDE) on gonaduct growth of larval tiger salamanders, *Ambystoma tigrinum*. *Gen. Comp. Endocrinol.* **109**:94–105.
- Colborn T, Clement C.** 1992. Chemically induced alterations in sexual development: the wildlife/human connection, *In* *Advances in Modern Environmental Toxicology*, Vol. 21, Colborn T, Clement C (ed), Princeton Scientific, Princeton, NJ.
- Colborn T, Dumanoski D, Myers. JP.** 1996. *Our stolen future*. Penguin Books, New York.
- Danzo BJ.** 1997. Environmental xenobiotics may disrupt normal endocrine function by interfering with the binding of physiological ligands to steroid receptors and binding proteins. *Environ. Health Perspect.* **105**:294–301.
- Fairbrother A, Ankley GT, Birnbaum LS, Bradbury SP, Francis B, Gray LE, Hinton D, Johnson LL, Peterson RE, Van Der Kraak GJ.** 1999. Reproductive and developmental toxicology of contaminants in oviparous animals, p. 283–361. *In* DiGiulio R, Tillitt DE, *Reproductive and developmental effects of contaminants in oviparous vertebrates*. SETAC Press, FL.
- Goldstein JA, Safe S.** 1989. Mechanisms of action and structure-activity relationships for the chlorinated dibenzo-p-dioxins and related compounds. *In* Kimbrough RD, Jensen AA (ed.), *Halogenated biphenyls, naphthalenes, dibenzodioxins and related compounds*, 2<sup>nd</sup> edition. Elsevier–North Holland, Amsterdam.
- Gorbman A, Dickhoff WW, Vigna SR, Clark NB, Ralph CL.** 1982. *Comparative endocrinology*. Wiley-Interscience.
- Griffin JE, Ojeda SR.** 1992. *Textbook of endocrine physiology*, 2<sup>nd</sup> edition. Oxford University Press, New York.
- Guillette LJ, Crain DA, Rooney AA, Pickford DB.** 1995. Organization versus activation: the role of endocrine-disrupting contaminants (EDC) during embryonic development in wildlife. *Environ. Health Perspect.* **103(Suppl 7)**:157–164.
- Hadley ME.** 1996. *Endocrinology*, 4<sup>th</sup> ed. Prentice-Hall, Inc., NJ.
- Harmon M, Boehm ME, Heyman RA, Mangelsdorf DJ.** 1995. Activation of mammalian retinoid X receptors by the insect growth regulator methoprene. *Proc. Natl. Acad. Sci.* **92**:6157–6160.
- Kelce WR, Monosson E, Gamcsik MP, Laws SC, Gray LE.** 1994. Environmental hormone disruptors: evidence that vinclozolin developmental toxicity is mediated by antiandrogenic metabolites. *Toxicol. Appl. Pharmacol.* **126**:276–285.
- Kelce WR, Stone CR, Laws SC, Gray, LE, Kemppainen JA, Wilson EM.** 1995. Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. *Nature* **375**:581–585.
- Kendall R, Dickerson R, Giesy J, Suk W.** 1998. *Principles and processes for evaluating endocrine disruption in wildlife*, Kendall R, Dickerson R, Giesy J, Suk W (ed.). SETAC Press, FL.
- Laws SC, Carey S, Kelce WR, Cooper R, Gray LE.** 1996. Vinclozolin does not alter progesterone receptor (PR) function *in vivo* despite inhibition of PR binding by its metabolites *in vitro*. *Toxicology* **112**:173–182.
- Le Drean Y, Kern L, Pakdel F, Valotaire Y.** 1995. Rainbow trout estrogen receptor presents an equal specificity but a differential sensitivity for estrogens than human estrogen receptor. *Mol. Cell. Endocrinol.* **109**:27–35.

- McMaster ME, Van Der Kraak GJ, Munkittrick KR.** 1996. An evaluation of the biochemical basis for steroid hormone depressors in fish exposed to industrial wastes. *J. Great Lakes Res.* **22**:153–171.
- Munkittrick KR, Van Der Kraak GJ.** 1999. Appropriate uses of physiological techniques for endocrine studies, p.95–118. *In* Henshel DS, Black MC, Harass MC (ed.), Standardization of biomarkers for endocrine disruption and environmental assessment, vol. VIII, Environmental toxicology and risk assessment. American Society for Testing and Materials.
- Nakhle AM, Rosner W.** 1996. Stimulation of prostate cancer growth by androgens and estrogens through the intermediacy of sex hormone-binding globulin. *Endocrinology.* **137**:4126–4129.
- National Research Council.** 1999. Hormonally active agents in the environment. National Academy Press, Washington, DC.
- Norris DO.** 1997. Vertebrate endocrinology, 3<sup>rd</sup> ed. Academic Press, Inc. California.
- Norris DO.** 2000. Endocrine disruptors of the stress axis in natural populations: how can we tell? *Amer. Zool.* **40**:393–401.
- Ono M, Kawauchi H.** 1994. The somatolactin gene, p. 159–174. *In* Farrell AP, Randall DJ (ed.), Fish physiology, vol. XII. Academic Press, Inc., CA.
- Peterson RE, Theobald HM, Kimmel GL.** 1993. Developmental and reproductive toxicity of dioxins and related compounds: cross-species comparisons. *Crit. Rev. Toxicol.* **23**:283–335.
- Peterson RE, Cooke PS, Kelce WR, Gray LE.** 1997. Environmental endocrine disruptors, p.181–191, vol. X. *In* Comprehensive toxicology. Elsevier Sciences, New York.
- Petit F, Valotaire Y, Pakdel F.** 1995. Differential functional activities of rainbow trout and human estrogen receptors expressed in the yeast *Saccharomyces cerevisiae*. *Eur. J. Biochem.* **233**:584–592.
- Preziosi P.** 1998. Endocrine disruptors as environmental signallers: an introduction. *Pure and Appl. Chem.* **70**:1617–1631.
- Rand-Weaver M, Noso T, Muramoto K, Kawauchi H.** 1991. Isolation and characterization of somatolactin, a new protein related to growth hormone and prolactin from Atlantic cod (*Gadus morhua*) pituitary glands. *Biochemistry* **30**:1509–1515.
- Rolland RM, Gilbertson M, Peterson RE** (ed.). 1997. Chemically induced alterations in functional development and reproduction of fishes. Proceedings from a session at the Wingspread Conference Centre. SETAC Press, FL.
- Rosner W.** 1990. The functions of corticoid-binding globulin and sex hormone-binding globulin: recent advances. *Endocrine Rev.* **11**:80–91.
- Safe S.** 1995. Modulation of gene expression and endocrine response pathways by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Pharmacol. Ther.* **67**:247–281.
- Shelby MD, Newbold RR, Tully DB, Chae K, Davis VL.** 1996. Assessing environmental chemicals for estrogenicity using a combination of *in vitro* and *in vivo* assays. *Environ. Health Perspect.* **104**:1296–1300.
- Van Der Kraak GJ, Wade MG.** 1994. A comparison of signal transduction pathways mediating gonadotropin actions in vertebrates, p.59–63. *In* Davey KG, Peter RE, Tobe SS (ed.), Perspectives in comparative endocrinology. National Research Council of Canada, Ottawa.
- Van Der Kraak GJ.** 1998. Observations of endocrine effects in wildlife with evidence of their causation. *Pure and Appl. Chem.* **70**:1785–1794.
- Van Der Kraak GJ, Zacharewski T, Janz D, Sanders B, Gooch J.** 1998a. Comparative endocrinology and mechanisms of endocrine modulation in fish and wildlife. *In* Kendall RJ, Dickerson RL, Giesy JP, Suk WA (ed.),

- Principles and processes for evaluating endocrine disruption in Wildlife. SETAC Press, FL.
- Van Der Kraak GJ, Chang JP, Janz DM.** 1998b. Reproduction. *In* Evans DH (ed.), The physiology of fishes, CRC Press, New York.
- Van Der Kraak GJ, Hewitt LM, Lister AL, McMaster ME, Munkittrick KR.** 2001. Endocrine toxicants and reproductive success in fish. *Hum. Ecol. Risk Assess.* In press.
- Zacharewski T.** 1997. *In vitro* bioassays for assessing estrogenic substances. *Environ. Sci. Technol.* **31**:613–623.