ENDOCRINE DISRUPTING CHEMICALS and ENDOCRINE ACTIVE AGENTS

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RESEARCH WORK ORDER NO. 193

RELATIONS OF ENVIRONMENTAL CONTAMINANTS, ALGAL TOXINS, AND DIET WITH THE REPRODUCTIVE SUCCESS OF AMERICAL ALLIGATORS ON FLORIDA LAKES

SEPTEMBER 2001

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INTRODUCTION

Since the early 1990's, it has been established that a wide variety of anthropogenic (man-made) chemicals in the environment are capable of modulating and/or adversely affecting or disrupting endocrine function in vertebrate organisms (see recent reviews: [1, 2 3-13]). The physiological effects of exposure to these chemicals has been termed "endocrine disruption" and the active compounds labeled as "endocrine-disruptors" or "endocrine-active-agents". Endocrine disruption has been defined by the US-EPA[12] as the action of "an exogenous agent that interferes with the production, release, transport, metabolism, binding, action, or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes". This definition was further expanded by the US-EPA Endocrine Disruption Screening and Testing Advisory Committee (EDSTAC)[14] to indicate that these effects are "adverse" and may involve a wide assortment of endocrine mediated functions and potential receptor mediated events. Indeed, effects may involve the steroid receptor superfamily, including the sex steroids, thyroid hormones and adrenal hormones, as well as hypothalamic-pituitary and other protein hormones.

The physiological processes regulated by the endocrine system are diverse and numerous. Likewise, the mechanisms of action and effects of potential endocrine disrupting chemicals (EDCs) are equally diverse (see Figure 1). Receptor-mediated events involve EDC's acting as hormone mimics (agonists and/or antogonists) and adversely impacting hormone synthesis, catabolism, secretion, transport and/or signal transduction. Examples of

non-receptor mediated modes of EDC action include altered enzyme function and selective toxicities for endocrine active or target tissues whereas altered gene expression and induction of oxidative stress are types of receptor mediated events. EDCs may also act by altering developmental processes often producing multigenerational effects.

Endocrine-active anthropogenic chemicals are also numerous and diverse. (see reviews [1-13]) Evidence for endocrine-disrupting effects due to these chemicals comes from a diverse array of reports involving multiple vertebrate taxonomic groups, limited invertebrate taxa, and results from both in vitro and in vivo studies. Reported effects of EDCs have included effects at multiple levels of biological organization, including molecular, biochemical, cellular, tissue, organism, community and population. However, few reports have documented effects at the community level and higher. In addition, most studies have focused upon reproductive effects, however, effects on growth, metabolism, and thyroid and immune function have also been noted. Today most evidence has also been derived from studies of wildlife and the ecotoxicology of potential EDCs and/or endocrine-modulating chemicals. This chapter summarizes the current evidence for the endocrine-disrupting effects of specific chemicals and chemical classes in vertebrate wildlife with a discussion on potential mechanisms/modes of action.

1.1 GENERAL and COMPARATIVE ENDOCRINOLOGY

To fully understand the mechanisms by which anthropogenic and/or natural EDCs may modulate endocrine function, normal functioning of the endocrine system must be understood. Indeed, an assessment of the risk of potential EDC exposures and effects requires critical information from a variety of disciplines, including endocrinology, and an understanding of the variation among and within vertebrate classes. The following section is a brief overview of vertebrate endocrinology and the hormones that may be involved in endocrine modulation or disruption.

Figure 1 summarizes the hypothalamic-pituitary-gonadal axis for fish as an example of the endocrine system, its diverse control over reproductive and developmental processes, and sites at which EDCs may exert endocrine-disrupting effects. In general, this model is also applicable to other oviparous vertebrate species, including birds, amphibians and reptiles.

The endocrine system is a collection of hormone-secreting cells, tissues, and ductless glands (e.g. the pituitary, thyroid, adrenal, and gonads) that play an especially important role in growth, development, reproduction,

and homeostasis. Tissues of the endocrine system synthesize and secrete chemical messengers, hormones, that influence virtually every stage of the life-cycle of an organism, from gametogenesis and fertilization, through development into a sexually mature organism. Endocrinology is the study of tissues that secrete hormones into the blood and the subsequent effects hormones have on target tissues. Hormones are released into the extracellular environments and affect either neighboring cells (paracrine control), the emitting cell (autocrine control), or other target tissues (endocrine control). Some nerve cells also release hormones into the blood (neuroendocrine control) or into extracellular fluid for communication with other nerve cells or non-nerve cells (neurotransmission). Pheromones are hormones secreted into the external environment for communication with other individuals or species. In addition, there are several hormones, which act through more than one of these chemical-signaling modes.

The vertebrate hypothalamus and the pituitary gland (or hypophysis) have an essential role in regulating endocrine and non-endocrine target tissues.[15-17] The hypothalamus and pituitary are functionally and anatomically linked, forming the hypothalamic-pituitary axis. In mammals, the pituitary is composed of four anatomically and functionally distinct regions: the adenohypophysial pars distalis and pars intermedia, and the neurohypophysial median eminence and pars nervosa. In fish, the pars distalis is additionally separated into two regions which contain different cell types and produce different hormones.[18] The pituitary gland of amphibians, birds and reptiles, is similar to the mammalian pituitary gland.[16] Indeed, the basic arrangement of the hypothalamic-pituitary axis is essentially the same in all vertebrate groups, with the exception of teleost fishes which lack a median eminence.[16]

The hypothalamus directly controls pituitary hormone secretion via the production and release of a number of peptide and nonpeptide hormones. These pituitary-tropic hormones are generally categorized as releasing hormones (RH) or release-inhibiting hormones (RIH) depending on their function. Hypothalamic hormones include: corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), gonadotropin-releasing hormone (GnRH), growth hormone-releasing hormone (GHRH), growth hormone release-inhibiting hormone (GHRIH, somatostatin), and prolactin release-inhibiting hormone (PRIH). Other hypothalamic hormones also include critical neurotransmitters such as catecholamine and dopamine.[19]

The principal neurohypophysial (neuro-pituitary) hormones in mammals are arginine, vasopressin and oxytocin. Birds, reptiles, and amphibians have structurally-related peptides: mesotocin and arginine vasotocin,[20]

while fish in general have arginine vasotocin and isotocin or mesotocin, depending on the species.[16] These hormones are critical for milk secretion, oviductal and uterine contraction, renal water absorption, and vaso-constriction and dilation. In all vertebrates, these neurohypophsial hormones are produced in the hypothalamus and are transported to the pituitary where they are stored until release into the bloodstream.

Hormones produced by the mammalian adenohypophysis are the pituitary-derived tropic hormones including growth hormone (GH), adrenocorticotropin (ACTH), melanotropin (MSH), thyroid-stimulating hormone (TSH), prolactin (PRL), and the gonadotropins: follicle-stimulating hormone (FSH), and luteinizing hormone (LH). Secretions of ACTH, TSH, and the gonadotropins (FSH and LH) are each regulated by negative feedback. Although structurally related counterparts for the adenohypophysial hormones have been identified in fish, amphibians, birds and reptiles,[16] there are important differences in hormone actions across vertebrate groups. For instance, prolactin is associated with reproduction and lactation in mammals but is an important osmoregulatory hormone in fish.[21] Although FSH and LH function similarly in mammalian and avian reproduction, reptiles do not synthesize an LH-like gonadotropin and instead utilize FSH to regulate gonadotropin-related functions.[15] In fish and amphibians, two different gonadotropins, GTH-I and GTH-II, have been identified that act similarly to mammalian FSH and LH, respectively.[17] Growth hormone (GH) generally regulates body and tissue growth, however, in non-mammalian vertebrates it is also involved in osmoregulation. In mammals and birds, ACTH is responsible for stimulating the production of corticosteroids by the adrenal gland, which in turn plays a role in metabolism, ion regulation and stress responses. The role of ACTH in fish and amphibians is less clear, however, and MSH may have similar properties in these taxonomic groups. Indeed, similarities in hormone structure may not necessarily represent similar hormone function in non-mammalian vertebrates.

Growth hormone is important for bone growth and as an anabolic hormone during development.[22] It has direct effects on a wide variety of tissues, as well as indirect effects which are modulated by growth factors, such as insulin-like growth factor-I (IGF-I).[22] In conjunction with thyroid hormones, GH is necessary for the development of a wide number of tissues ranging from cardiac[23] and skeletal muscle,[24] to bone[25] and brain development.[26] In non-mammalian species GH probably functions in a similar manner, however, less is known about growth hormone in fish, amphibians and reptiles.

The adrenal glands, thyroid gland, and gonads are all directly regulated by the pituitary gland.[16] Thyroid

hormones, produced by thyroid glands, and steroids produced by the adrenal cortex and gonads can indirectly inhibit their own secretion by inhibiting the release of pituitary and hypothalamic hormones (negative feedback). In response to TSH, the thyroid gland produces two hormones, triiodothyronine (T_3) and tetraiodothyronine (T_4). In mammals, T_3 and T_4 have important effects on metabolism and development.[16] Thyroid hormones also play an essential role in fish and amphibian metamorphosis. Indeed, thyroid hormones determine the timing of developmental processes, and metamorphosis is almost entirely controlled directly by thyroid hormones.[16, 27-29] Some metamorphic processes that are under the control of thyroid hormones include the migration of the eye and dorsal fin growth in fish,[30, 31] amphibian tail and gut resorption,[27, 32, 33] restructuring of the amphibian head ,[34, 35] amphibian limb development,[36] and amphibian gill resorption.[37] Thyroid hormones also have important roles during fish smoltification.[38-40]

The mammalian adrenal gland produces two important steroid hormones, aldosterone and corticosterone. Aldosterone plays an important role in the maintenance of sodium concentrations, and corticosterone is primarily involved in regulating blood glucose.[16] Adrenal steroids function similarly in birds, but very differently in other non-mammalian vertebrates. In amphibians, aldosterone and corticosterone are equally effective as regulators of blood glucose, whereas in fish and reptiles, corticosterone serves to regulate blood glucose and sodium. While adrenal hormones have critical roles in all vertebrates, characterizations of their functions in non-mammalian vertebrates are limited, and interspecies differences have not been thoroughly evaluated.

In all vertebrate classes, gonadal function is dependent upon the hypothalamic-pituitary axis through the production of GnRH and gonadotropins.[16] In mammals, the gonadotropins include FSH and LH, which control different gonadal events. In females, FSH promotes ovarian follicular growth and LH induces ovulation. Both gonadotropins are also required for normal estrogen synthesis: LH stimulates the synthesis of androgens and FSH stimulates aromatization of androgens to estrogen. In males, FSH promotes spermatogenesis and LH promotes steroidogenesis and spermiation. The mammalian gonad also produces the peptide hormone inhibin which feeds back to inhibit FSH production. In both males and females, the pulsatile release of GnRH is regulated by the feedback of high circulating levels of androgens and estrogens. In birds, gonadotropins function in a similar manner, however, reptiles do not synthesize an LH-like gonadotropin and utilize FSH to regulate all gonadotropin-related functions.[15] In fish and amphibians, two different gonadotropins, GTH-I and GTH-II have been identified

and they act similarly to mammalian FSH and LH, respectively. GTH-I is involved in gonadal development, gamete production, and vitellogenesis, a process that involves the hepatic synthesis of yolk protein precursors (vitellogenin).[16, 17] GTH-II stimulates the final stages of oocyte maturation, as well as ovulation in females and spermiation in males. In general, gonadotropins exert effects on vertebrate gonads by binding to specific receptors. The primary gonadal response to gonadotropins is the synthesis and secretion of assorted sex steroids. In all vertebrates, the primary reproductive sex steroids include androgens [e.g. testosterone (T), 11-ketotestosterone (11KT), androstenedione (A), dihydrotestosterone (DHT)], estrogens [estradiol (E_2), estrone (E_1), estriol (E_3)], and progestins [progesterone (P_4), dihydroxyprogesterone (DHP)]. Gonadal steroid hormones are involved in every aspect of reproduction from sex determination to the control of courtship behaviors and the development of secondary sex characteristics. Sex steroids also play an important role in brain development. For example, in mammals, E_2 and DHT, are involved in normal sexual differentiation of the brain.[41-43] Although reproductive function is regulated and modulated by sex steroids in all vertebrates,[16, 28, 44-47] there are distinct functional differences that must be noted. Indeed, functional differences in sex steroids are most evident for fish, amphibians, and reptiles with significant differences also existing within each of these taxonomic classes.[17] For instance, the primary androgen for spermatogenesis in mammals, birds, and reptiles is T, but in many fish and some amphibians the critical androgen for spermatogenesis is 11KT. Preliminary results from our laboratory would suggest that 11KT might not be the predominant androgen in live-bearing fish.(such as mosquito fish *Gambusia affinis*). E_2 is the sex steroid responsible for oocyte growth and maturation in all vertebrates, however, estradiol also regulates and induces the synthesis of vitellogenin (VTG) in oviporous vertebrate species.[16, 17, 48, 49] Vitellogenin is the primary protein egg yolk precursor produced by the liver and which production is stimulated by estrogens. Progestins are critical to pregnancy in mammals, but function in reptiles and birds in post-ovulatory events such as the regulation of eggshell deposition. In fish, progestins are responsible for final egg maturation prior to oviposition. Gonadal sex steroids can also have dramatic effects on sex differentiation in fish, amphibians and reptiles, effects which are not observed in birds nor mammals.[17, 28, 50] When applied early during development, sex steroids can cause sex reversal in fish, amphibians, and reptiles. Therefore, the genetic sex of the individual can be different from the phenotypic sex. Finally, the effects of sex steroids on gonadal differentiation and sex reversal vary dramatically between species and across developmental stages, and therefore these differences need to be noted and considered in any study of

potential EDC effects in vertebrate wildlife.

1.2 MECHANISMS OF ENDOCRINE MODULATION

There is significant evidence to suggest that a wide variety of anthropogenic chemical contaminants in the environment, can disrupt or modulate endocrine function in a wide variety of vertebrate and some invertebrate organisms. However, information regarding the mechanisms that lead to these endocrine modifications is limited. It is, nonetheless, critical that mechanisms and modes of action for EDCs and/or endocrine-active agents be understood. Mechanisms of action are generally difficult to elucidate and are complicated by multiple factors including chemical properties, routes of exposure, as well as endocrine system and species-and tissue-specific physiological differences. Furthermore, the integration of the nervous, endocrine, reproductive, hepatic, and other target systems, as well as multiple feedback regulatory pathways, add to the complexity of understanding EDC mechanisms (see Figure 1 as an example).

Potential mechanisms of action for EDCs are diverse. EDCs may interrupt multiple pathways along the hypothalmic-pituitary-target tissue axis, potentially disturbing the normal synthesis, transport, release, binding, action, or elimination of natural hormones in the body.[1-13] EDCs may alter the hypothalamic-pituitary axis, which can have wide spread effects through the disruption of endocrine functions downstream of the hypothalamus. There is increasing evidence that EDCs may disrupt endocrine function by influencing the regulation/release of the pituitary-tropic hormones. Indeed, polychlorinated byphenyls (PCBs) have been shown to interfere with the neurotransmitters that control GnRH secretion resulting in decreased GnRH production as well as subsequent reductions in gonad size and plasma concentrations of sex steroids.[51] In contrast, cadmium results in both increased GnRH secretion and increased gonad size and plasma sex steroids. In mammals, neonatal exposure to diethylstilbestrol (DES) or dichlorodiphenyltrichloroethane (DDT) results in both reduced GnRH and LH production.[52] These results demonstrate that interference at one site along the hypothalmic-pituitary axis can affect multiple downstream events. Furthermore, the hypothalmus and pituitary are regulated by the feedback of hormones from several other endocrine active tissues; therefore, alterations in different hormone concentrations can also affect hypothalmic and pituitary function.

EDCs can also exert effects and disrupt the function of other endocrine tissues and hormones downstream

of the hypothalmus and/or pituitary. Hormones are synthesized by specific endocrine tissues, secreted into the bloodstream, and transported by binding proteins to target tissues to interact with receptors, elicit responses, and be metabolized or degraded. EDCs can block or enhance the function of hormones by interfering with any one or several of these critical steps. For instance, EDCs may interfere with hormone synthesis[28, 53] therefore altering endocrine activity by directly affecting the availability of specific hormones and/or critical precursors. Failure to synthesize appropriate hormones can result from either an alteration in the biosynthetic enzymes and/or in the availability of precursor molecules. The initial, as well as rate-limiting, step in the biosynthesis of hormones may often be affected. EDCs can inhibit the uptake of critical precursors and the subsequent conversion to hormone products.[54-56] For example, the cytochrome P450 (cyp 450) monooxygenases constitute a super family of enzymes that play essential roles in both the synthesis (steroidogenesis) and metabolism of steroid hormones. Many of these enzymes appear to be sensitive to EDCs. [53, 57-60] EDCs can affect the number or activity of specific monooxygenases, thereby affecting the rate of hormone metabolism and clearance. Since specific P450 enzymes, such as CYP1A, are also responsible for metabolizing foreign compounds, such as EDCs, EDC stimulation of CYP1A and /or other monooxygenases that hydroxylate them prior to their elimination may in turn contribute to increased clearance of sex hormones by inducing other monooxygenase activities.[61] EDCs have also been reported to increase the activity of several other microsomal enzymes including aminopyrine demethylase, glucuronyl transferase, and p-nitroreductase.[62, 63] Some EDCs may also induce hormone-like effects due to a resistance to degradation. For example, many synthetic hormones such as ethynyl estradiol (EE_2), a synthetic estrogen used in birth control pills, are not degraded readily by the enzymes that normally metabolize the endogenous hormones.[64] EDCs can also interfere with the binding of hormones to transport proteins, preventing their delivery to target tissues.[65, 66] The absence of available binding proteins may result in both faster uptake or increased degradation of free-circulating hormone.[67-69] For example, the sex hormone binding globulin (SHBG) has high affinity for both T and E_2, which is necessary to prevent degradation and clearance of these hormones, as well as enable their transport to target tissues.[70] EDCs, which mimic estrogens or androgens, may bind to these globulin proteins and displace the endogenous sex steroids, thereby increasing the elimination rates for endogenous hormones. Although, several studies suggest that globulins may also facilitate the transport of EDCs to target tissues,[70] the greater binding affinity of globulins for endogenous hormones probably limits this process.[71]

EDCs may also bind to hormone receptors and either activate (agonize)[72-74] or inhibit (antagonize)[75] receptor function. Indeed, many studies have focused upon EDCs as hormone-mimics and the potential for these compounds to interact with hormone-specific receptors.[75] Potential EDCs have been evaluated extensively for their ability to bind to the estrogen receptor (ER). Estrogens normally bind to the ER located in the nucleus of target cells. The E_2-bound ER has a high affinity for DNA sequences called estrogen response elements (ERE). After binding the ERE, the ER-DNA complex interacts with various transcription factors, chromosomal proteins, and regulatory factors in order to induce or inhibit the transcription of specific genes and enable endocrine specific response. EDCs can block or enhance the function of a hormone or endocrine target tissue by interfering with any one or several of these critical steps. Although the potential estrogenic activities of EDCs have overshadowed studies of other receptor mediated EDC activities, EDCs that act as androgens or anti-androgens via interaction with the androgen receptor (AR) have also been noted.[75-77] Unlike the ER, which has an estrogen specific response element, the response element for the AR is shared with other steroid receptors, including the glucocorticoid (GR), progesterone (PR), and mineralocorticoid (MR) receptors. The consequences of this is that EDCs which have androgenic activities may exert broader effects than those attributed to a simple androgen mimic. EDCs may also interact with a wider variety of receptors important for endocrine function. For example, some EDCs (e.g. TCDD and other planar hydrocarbons) are reported to have antiestrogenic activities by interacting with the aryl hydrocarbon receptor (AhR) rather than by competitively binding to the ER. The AhR is an intracellular receptor, expressed by many different cell types, and which functions as a transcription factor.[78, 79] EDC interactions with the AhR may interfere with estrogen responses in a number of ways: by reducing estrogen ER binding;[80] by blocking the binding of the ER to the ERE;[81] by impairing nuclear translocation;[82] and/or by suppressing gene transcription.[83] These examples demonstrate the varied receptor mediated activities of EDCs.

Endocrine-disrupting effects may also occur due to direct or indirect toxicities for specific endocrine active or target tissues. For example, many lipophilic EDCs will primarily accumulate in fatty tissues, such as the liver and gonads, interfering with the synthesis and mobilization of lipids and thereby inhibiting specific endocrine related functions, such as vitellogenesis. It is important to point out, however, that specific mechanisms or modes of action for most EDCs currently identified are not well elucidated nor understood. This stems from the fact that mechanisms are often difficult to identify and are complicated by multiple factors including differences in EDC

specific properties, routes of exposure, and vertebrate class and species differences. Nonetheless, it is critical that mechanisms of action for EDCs and/or endocrine-active agents be understood in order that effects in wildlife be prevented and that appropriate screening and testing methods be developed.

2 SCREENING AND MONITORING FOR ENDOCRINE DISRUPTORS

Analytical methods have long been used to determine concentrations of chemical residues that persist in the environment (e.g., water, sediment) and accumulate in biota (e.g., tissue and body burdens). Although these approaches are useful for characterizing the presence and distribution of specific EDCs in the environment they fail to indicate whether chemical exposures have biological consequences. The development of EDC-specific screening and monitoring procedures aid in the establishment of potential relationships between environmental EDC concentrations and biological responses. These procedures rely upon mechanism specific in vitro and in vivo procedures, which measure biological responses. In the past decade, several in vitro and in vivo assays have been proposed that can be used to screen or monitor both individual EDCs, specific EDC mixtures, and/or complex environmental mixtures for potential endocrine disrupting or modulating activity.[76]

2.1 IN VITRO ASSAYS

Several in vitro assays have been described for evaluating potential endocrine disrupting or modulating activities of EDCs.[76] These assays are based upon several specific mechanisms of action for EDCs, including receptor binding, gene expression, cell proliferation, and cell differentiation.[84] Advantages of in vitro systems include low cost, high reproducibility, and the rapid analysis of large numbers of samples. These assays are also valuable for studying mechanisms of action of compounds, screening effects of mixtures, and for detecting potential interaction effects. Results from these screening procedures can aid in the subsequent development and validation of assays. In vitro assays, however, generally lack ecorelevance because pharmacokinetics, biotransformation, and binding to carrier proteins may not be accurately represented. For example, some EDCs are activated or deactivated in vivo by enzymatic conversion during metabolism, conjugation, and/or excretion. These limitations must be considered when interpreting or applying results from in vitro screening tests.

Receptor binding assays can be utilized to screen for and identify potential EDCs, which function via

receptor-mediated pathways, since they can evaluate whether specific EDCs can bind to specific receptors. Depending on the receptor of interest, receptor-binding assays utilize either crude cell fractions, such as plasma membranes, cytosol, or the nucleus. Cell fractions may be obtained from specific vertebrate organisms or from established cell lines, transformed cells[85, 86] or transfected cells.[87] Although in vitro receptor binding assays are relatively simple and inexpensive to conduct, they do not necessarily reflect binding under in vivo conditions, and are of little use in screening for EDCs that operate by non-receptor mediated pathways. Finally, these assays do not differentiate between agonist and antagonist properties.

Additional in vitro assays have utilized the ability of EDCs to induce target-cell-specific proliferation and/or differentiation. For instance, MCF-7 cells, derived from human breast cancer cells, have been widely utilized for the development of the E-screen assay, which evaluates the ability of specific EDCs or EDC mixtures to both bind and express the ER[88] and the resultant cell proliferation as a response.[89-95] EDCs are identified as potential E_2 agonists if there is a significant increases in cell proliferation and cell proliferation, which in turn, is quantified by counting cell nuclei[93] or measuring other responses, such as metabolic reductions. Although the E-screen assay has been extensively used as a screen for estrogenicity[77, 92, 93, 96] a positive response cannot be necessarily interpreted as an indicator for the presence of E_2 agonists. In addition, ER antagonists and anti-androgens are not detected using this assay, and thus a significant number of false negatives are common. Before a compound is identified as an EDC, positive responses with the E-screen assay should be confirmed by in vivo studies.

A number of additional, in vitro, cell-based expression assays have also been developed to measure receptor-dependent biological responses. Expression assays evaluate the induction or suppression of proteins by specific genes in response to potential receptor mediated EDCs and or mixtures. Measured protein endpoints for these receptor specific expression assays include: Vitellogenin[72-74, 95, 97, 98] sex hormone binding globulins;[99] luciferase;[100] galactosidase;[101] and chloramphenicol acetyltransferase.[87] However, these assays are general and are not limited to the action of EDCs. Additional cell types/lines have also been utilized for in vitro expression assays, including fish hepatocytes,[72-74, 95, 99] MCF-7,[96, 102] HeLA,[87, 99] and yeast.[99, 102] The types of cells used in expression assays are critical to any interpretations. Indeed, significant differences in responses between yeast cell-based assays and mammalian cell assays have been reported[99] and sensitivities vary greatly[101] Nonetheless, expression assays have several advantages as compared to other in vitro screening assays. Unlike receptor binding or cell

proliferation assays, expression assays can be used to detect both agonists and antagonists.[87, 100, 103] Expression assays can also evaluate potential EDCs that influence many aspects of gene expression in addition to those that operate through receptor-mediated functions. Nonetheless, in vitro expression assays generally have high variability and lack ecorelevance.

2.2 IN VIVO ASSAYS

The effects of EDCs occur at many biological levels of organization, including molecular, biochemical, organelle, cell, tissue, organism, population, community, and ecosystem. The use of a battery of biomarkers which reflect multiple biological levels of organization would enable a more thorough evaluation of both exposure and the potential mechanisms of action. Although responses at the population level and higher are the most biologically ecorelevant, they are rarely utilized as biomarkers since these responses are complex, less specific, and require greater effort and time. Indeed, most of the current biomarkers are limited to the measurement of responses at the molecular, biochemical, cellular, and/or organism levels. These biological responses or biomarkers of effects and/or exposure are widely utilized for the assessment and identification of potential EDCs.

In vivo assays for the identification of EDCs are not mechanism dependent and provide results which are more ecorelevant than in vitro assays. Indeed, in vivo assays rely upon either natural exposures or controlled exposures based on expected or predicted environmental exposures. In vivo assays for EDCs can detect effects on endocrine function, regardless of the mechanism of action, as well as identify a potential EDC which would not necessarily exhibit activity in an in vitro screening assay. Most importantly, in vivo screening assays both identify potential EDCs and enable the description and evaluation of potential effects.

In vivo assays for EDCs may involve the utilization of specific endocrine biomarkers as a way to evaluate potential effects. Widely used endocrine endpoint based in vivo assays have included: the uterotropic assay; the Hershberger assay; and the thyroid function assay. Although these assays were not originally designed for the evaluation and/or identification of EDCs, they have demonstrated the utility of in vivo assays for the identification of potential EDCs. The uterotropic assay utilizes prepubertal or adult ovariectomized female rats to assess uterine weight and histological responses to potential EDCs. The Hershberger assay evaluates androgenicity using androgen-dependent tissue (e.g. prostate and seminal vesicles) responses to potential EDCs. The thyroid gland

function assay evaluates potential EDC exposures and the subsequent evaluation of plasma concentrations of T3, T4, and TSH.

Biomarkers that detect alterations at the biochemical and/or molecular levels are frequently utilized for in vivo EDC screening assays.[104] Biochemical and molecular responses are generally the first detectable responses to an environmental change or stressor and can serve as early indicators of both exposure and effect. Effects at the molecular or biochemical level are highly sensitive and in some cases can be predictive of responses at higher levels of organization (tissue and organism levels). Examples of molecular-based in vivo EDC screening assays include, receptor analyses , transcriptional based analyses , and differential display.[14] These assays are, in general, based upon an analysis of specific molecular parameters for tissues collected following either natural or experimental exposures to potential EDCs. Although molecular-based in vivo assays are highly sensitive, they are difficult to validate and often lack ecorelevance. Examples of current biochemical-based in vivo EDC screening assays include: measurement of VTG production[105] and systemic hormone concentrations (e.g. plasma sex steroids, T_3, and T_4). Hormonal factors which control endocrine functions, have been characterized for mammalian and non-mammalian species.[17] In addition, systemic concentrations of various hormones, have been frequently utilized as biomarkers for EDCs in fish,[106-112] birds, reptiles, mammals, and amphibians. Indeed, these procedures have broad application to all vertebrate classes since hormones, especially the steroid and thyroid hormones, are evolutionarily conserved across all vertebrate classes. However, it must be noted, that the same hormones may differ in function significantly between and within vertebrate classes. For example, the primary androgen for spermatogenesis in mammals, birds, and reptiles is T, but in many fish and some amphibians, the critical androgen for spermatogenesis is 11KT. Vitellogenin has been utilized as a bioindicator of potential exposure and/or effects of estrogenic EDCs in fish and other oviparous vertebrates.[97, 98, 113, 114] This phospholipoprotein is one of several proteins under the control of E_2 and naturally induced in oviparous female fish, amphibians, birds, and reptiles.[115] Oviparous species have vitellogenic cycles which correspond to egg production. Potential EDCs, which mimic or alter endogenous E_2, may induce the expression of VTG. This assay has, in general, focused on responses in males, which do not exhibit clear vitellogenic cycles. However, it must be noted, that low background levels of VTG are likely to be normal in males and thus an identification of a potential EDC by this method cannot be solely based upon the presence of detectable VTG, but rather in a species-specific VTG response, which is significantly increased above background levels.

Additional in vivo EDC screening assays involve endpoints based on responses at the tissue and organism levels. Although these assays may have higher biological and ecological relevance, they are more variable and often specific to vertebrate classes or species. Examples of screening assays which rely upon tissue level responses include tissue somatic indices (e.g. GSI), tissue histopathology , altered secondary sex characteristics,[116-120] and sperm quality assessments.[95] In vivo assays which rely upon organism responses may include, assessments of egg numbers/ovarian development,[108, 120-123] sexual maturity,[120] neonatal/embryonic mortality,[121-126] reproductive impairment,[110, 127] and evaluation of egg hatchabilities,[121, 126, 128, 129]and nest numbers.[124, 126, 129] Population and ecosystem endpoints of reproductive success may include, pod size, age class analyses, and population numbers.[130-132]

Valid in vivo screening procedures should provide information about EDC exposure and be indicative of expected or predicted physiological effects. In addition, in vivo biomarkers reflect the complex pharmacokinetic and metabolic factors that can affect EDC uptake and metabolism. In vivo assays and endpoints are influenced by both physiological and environmental variables, which make it difficult to establish clear cause-effect relationships between responses and specific EDCs. Nonetheless, these assays are often the most useful for evaluating potential EDC effects and for the identification of environmentally relevant EDCs.

3.0 EDC EFFECTS: EVIDENCE FOR SPECIFIC CHEMICALS AND CHEMICAL CLASSES

The previous section reviewed many of the possible mechanisms by which environmental contaminants may alter endocrine function in wildlife and fish. The following section introduces several classes of environmentally relevant contaminants with reported or potential endocrine-disrupting activity in vertebrates. This review presents evidence for EDC effects for several specific chemical classes: polycyclic aromatic hydrocarbons, polychlorinated biphenyls and polybrominated biphenyls, dioxins, organochlorine and non-organochlorine pesticides, complex environmental mixtures, and metals. For most chemicals, the specific mechanism of action is not well understood, nor does chemical structure necessarily indicate nor suggest endocrine functionality, mimicry or EDC activity, (see Figure 2 as an example of chemical structures for several environmental estrogens). In fact, direct evidence of endocrine activity is often difficult to demonstrate and thus is generally absent. This review includes reports from a variety of laboratory and field studies that have explored the effects of EDCs in wildlife,

and discusses the potential or suspected MOA (see Table 1).

3.1 Polycyclic aromatic hydrocarbons (PAHS):

Polycyclic aromatic hydrocarbons occur naturally as products of incomplete combustion of organic compounds and enter aquatic environments via oil spills, waste discharge, runoff, and dry or wet deposition. Although they are biodegraded in soils and water within weeks to months, the metabolites are often longer lasting and more toxic. PAHs are common in sewage effluent, with average concentrations of 50 pptr, for the naphthalenes such as benzo a pyrene (BaP). The Great Lakes represents one of the most PAH contaminated regions in the world, with concentrations in the hundreds of pptr. Additional PAH sources include pulp and paper mills, where concentrations of PAHs can be as high as 30 ppb.

Birds can be exposed to PAHs through ingestion of contaminated food and water, or through the skin in cases of oil spills. Petroleum hydrocarbons can also be absorbed through the shell.[133] In a review by Hoffman[134] PAHs applied to the shells of eggs caused mortality and reduced hatchability. In studies reviewed by Fry,[135] exposure to petroleum oil increased circulating corticosterone levels and alters reproduction through negative feedback to the hypothalamus-pituitary-gonadal (HPG) system. Yolk formation may also be depressed after exposure to oil resulting in a reduction in egg numbers.[136] Exposure to as little as 0.1 - 2.0 ml weathered crude oil interferes with egg production, laying, incubation, and pair bonding. Field exposure of adult storm petrels *(Oceanódroma sp.)* with dependent chicks, reduced foraging and feeding of chicks, resulting in reduced growth or death.[137]

Population studies with pigeon guillemonts *(Cepphus columba)* after the Exon Valdex spill indicated a decline in numbers for three consecutive years but no effects on reproduction,[138] however, reproductive effects in the black oystercatcher (*Haematopus bachmani*) were noted.[139] There was a decrease in non-breeding pairs, a decrease in egg size, and higher chick mortality, which was directly related to the amount of oil present in the foraging territory. Birds exposed to oil may exhibit changes in adrenal hormone synthesis and elevated hepatic mixed oxidase activity, which may increase metabolic clearance of corticosterone.[133] In a laboratory study female mallards (*Anas platyrhynchos*) that ingested crude oil hatched fewer live ducklings per pair.[133, 140] There was evidence of suppression of follicular development, eggshell thinning, decreased hatchability, and reduced levels of plasma E_2,

estrone, P, and LH in females. These results suggest that the oil acts on ovarian steroidogenesis, reducing positive feedback to the pituitary, and causing a decline in LH, a delay in ovarian maturation, and reduced fertility.

Laboratory and field studies present clear evidence for the adverse affects of PAHs in fish. Thomas et al., [141] have elucidated the impact of BaP on endocrine and reproductive activities in female Atlantic croaker (*Micropogonias undulatus*) and spotted seatrout (*Cynoscion nebulosus*). Atlantic croaker fed 0.4 mg BaP/70g/day for 30 days during the period of ovarian recrudescence experienced impaired ovarian growth with a concomitant reduction in plasma E_2 and T. GSI in control females increased 5-fold over the course of the study, whereas GSI of exposed females reached only 66% of controls. In vitro production of sex steroids was not impaired by BaP, and there appeared to be a relationship between the amount of ovarian tissue (i.e., size of ovaries) and steriodogenic capacity. Similar results were reported in a separate study of female Atlantic croaker exposed to BaP via injection for 30 d.[142] In this study in addition to reduced GSI and plasma sex steroids, a reduction in the number of hepatic ERs and plasma VTG was observed. BaP did not interfere with the binding of E_2 to the ER under in vitro competition studies and, again, there was no clear evidence for a direct effect of BaP on steroidogenesis. In vitro competition studies using hepatic ER from spotted seatrout supported early results, and concentrations of BaP ranging from 10^{-12} to 10^{-6} were unable to displace radiolabeled E_2 from the ER.[143] This is consistent with mammalian studies, and suggests that BaP must undergo metabolic activation in order to interact with the ER. The effects of the PAH 3-methylcholanthrene, on endocrine and reproductive function in ricefield eels (*Monopterus albus*) were similar to those observed for BaP-treated Atlantic croaker. exposure to 4 ppm 3-methylcholanthrene for 7 d resulted in reduced E_2, Te, and VTG, GSI, and altered ovarian histology.[144]

Several field studies have documented altered reproductive activity in fish residing in PAH-contaminated waters. For instance, gonadal development was impaired and E_2 concentrations were depressed in English sole from highly contaminated areas of Puget Sound, Washington. Reproductive impairment was statistically correlated with elevated PAH concentrations, as measured by the presence of fluorescent aromatic compounds (FACs) in the bile of fish.[145, 146] Other examples in which PAH exposure may have been related to endocrine alterations and/or reproductive dysfunction include altered ovarian development in plaice (*Pleuronectees platessa*) exposed to crude oil,[147] reduced GSI, increased liver size and ethoxyresorufin O-deethylase activity (EROD) in white sucker (*Catostomus commersori*) residing downstream of pulp and paper mills,[148] and decreased GSI in bream (*Abramis*

brama) inhabiting contaminated areas of the Rhine River.[149] Although PAH concentrations were abnormally high in the field studies described above, they were only one of a consortium of pollutants that may have caused the observed effects. Furthermore, several histological studies report no differences in the gonads of male and female fish from control and PAH-contaminated sites.[150, 151] A combination of field and laboratory experiments is still necessary before the reproductive alterations observed in the wild can be clearly attributed to PAH exposure.

PAHs known CYP450 inducers. For example, the PAH β-naphthoflavone induced the expression of CYP 4501A1 (the primary xenobiotic-metabolizing enzyme) and inhibited VTG synthesis in E_2-stimulated liver cells from rainbow trout (*Oncorhynchus mykiss*)[152]. However, β-naphthoflavone had no effect on vitellogenesis when incubated without E_2. The degree of CYP 4501A1 induction was directly related to the extent of VTG inhibition, which suggests that β-naphthoflavone may be acting as an antiestrogen via the Ah (Aromatic hydrocarbon) Receptor, the intracellular receptor involved in CYP4501A1 expression. The effect of β-naphthoflavone on vitellogenesis in vivo appears to be more complicated. When juvenile rainbow trout were treated with 0.5 ppm E_2 and 25 or 50 ppm of β-naphthoflavone, an inhibitory effect on VTG synthesis was observed; however, lower concentrations of β-naphthoflavone (5 or 12.5 ppm) appeared to potentiate E_2-stimulated VTG production.[152] Furthermore, reduced VTG synthesis by higher concentrations of β-naphthoflavone was correlated with a decrease in radiolabeled E_2 binding to the ER. These results suggest that β-naphthoflavone influences VTG synthesis by regulating ER function, although it is likely that the antiestrogenic activity of PAHs involves multiple mechanisms. Several investigators have proposed that CYP4501A1 inducing compounds affect sex steroid concentrations by increasing their catabolism.[153] Evidence from a recent study[154] however, suggests that PAHs may also interfere with steroid biosynthesis. Incubating vitellogenic ovarian tissue from female flounder (*Platichthys flesus*) with 3 PAHs (phenanthrene, BaP, and chrysene) decreased androstenedione and E_2 secretion. In addition, phenanthrene inhibited steroid conjugation, and it was concluded that these PAHs inhibited key steroidogenic enzymes, including CYP450 17, 20 lyase, which is responsible for converting C21 to C19 steroids.

3.2 Polychlorinated and Polybrominated biphenyls (PCBs and PBBs):

PCBs are a group of synthetic organic chemicals, formed by the chlorination of biphenyls, which include 209 individual compounds (congeners). These substances were manufactured for a wide range of industrial

applications, including use as hydraulic fluids, lubricants, plasticizers, and coolant/insulation fluids in transformers. Several chemical properties make these compounds both highly useful and potentially hazardous. For instance, their chemical stability makes them ideal for industrial activities involving high temperatures; however, this stability also renders them persistent in the environment. The majority of PCBs that enter the water adsorb to organic particles and sediments, although they are essentially non-biodegradable in soils and sediments.[155] Furthermore, they are hydrophobic, which makes them excellent lubricants, yet allows them to bioaccumulate in tissues and biomagnify as they are passed along the food chain. Concentrations of PCBs in fish at contaminated sites may range from ppb to ppm,[156] and one study involving PCB153 revealed a half-life exceeding 10 years in adult eels (*Anguilla rostrata*). The production of PCBs is currently banned or highly restricted, the use of certain mixtures permitted only under tightly regulated conditions. Nonetheless, PCBs originating from industrial wastes, accidental leaks or spills, and careless disposal, continue to be a source of pollution and environmental concern.

Many studies examining the health hazards of PCBs describe the effects of occupational exposure in humans and the physiological responses of mammals and birds that have consumed large quantities of contaminated fish. These studies provide strong evidence that PCB exposure can lead to the development of cancer; disturbances of the immune, hepatic, pulmonary, and nervous systems; and impaired reproduction and development. Many of these abnormalities are enhanced in the offspring, even if exposure occurs prior to conception. Responses are believed to be dependent on species, sex, age, and chemical structure. [157]

Laboratory studies with mink (*Mustela vison*) have established an association between PCB residues and reproductive effects in wildlife,[158] but there are no field studies linking PCBs with reproductive effects.[159] In one study in which mink were fed meat from cows contaminated with Aroclor 1254 concentrations as low as 0.87 - 1.33 ppm resulted in reproductive failure.[160] Other feeding studies have shown impaired reproduction in mink with fat concentrations of 13.3 ppm and reproductive failure at concentrations of 24.8 ppm.[161]

Field studies with big brown (*Eptisecus fuscus*) and little brown bats (*Myotis lucifugus*) suggested a correlation between PCB residue levels and reproductive toxicity,[162, 163] however captive studies do not support this link.[164] Studies with ringed seals (*Pusa hispida*) have found a relationship between fat PCB concentrations and uterine horn occlusions.[165] Later studies with ringed and gray seals (*Halichoerus grypus*), however, failed to detect any relationship between PCB levels and pregnancy or impairment of the uterine horns. [166] Other studies have

linked PCBs, PCDDs, and CDFS with abortions and premature pupping in California sea lions (*Aalophus californianus*);[167] tumors and decreased fecundity in Beluga whales (*Delphinapterus leucas*);[168] skeletal lesions in harbor (*Phoca vitulina*) and grey seals; [169, 170] and immunosuppression in harbor seals.[171] In a field experiment with common seals (*Phoca vitulina*) animals fed PCB contaminated fish had a significant reduction in reproductive success, however in this study it was difficult to separate out the influence of other possible factors and contaminants.[172, 173]

PCBs have been associated with embryonic mortality, deformities, and low reproductive success in many species of birds. In the Great Lakes, contaminated colonies of common terns *(Sterna hirundo)* have been shown to have low recruitment and reproduction of bald eagles (*Haliaeetus leucocephalus*) has been reduced below the level of a stable population.[174] Feeding studies have been done using chickens, ringed turtledoves (*Streptopelia risoria*), Japanese quail (*Coturnix coturnix japonica*), mourning doves (*Zenaida macroura*) and ring-necked pheasants (*Phasianus colchinus*). In three studies, chickens that received 10 - 80 ppm Aroclor 1248 in the diet had a reduction in hatching[175 176 177] however in another study a diet of 20 ppm Aroclor 1254 did not affect this parameter.[178] Aroclor 1242 in the drinking water at 50 ppm produced embryo mortality and teratogeneses.[179] Aroclor 1254 in the diet of ringed-turtle doves has increased embryonic mortality and decreased parental attentiveness[180] and depleted brain dopamine and norepinephrin.[181] Eggshell thickness was affected in mallard hens[182] but another study produced no effects.[183] Studies with screech owls (*Otus asio*)[184] and atlantic puffins (*Fratércula árctica*) [185] also produced no reproductive effects.

Field studies have indicated PCBs as the cause of mortality of ring-billed gulls (*Larus delawarensis*) in southern Ontario[186] as well as the cause for increased embryo/chick mortality and reduced hatching success.[187, 188] PCBs have also been blamed for the low reproductive success and eggshell damage in Lake Michigan herring gulls (*Larus argentatus*).[189, 190] Reproductive success of Forster's terns (*Stérna. forsteri*) from a Green Bay colony was 52 % of that from inland colonies [191, 192] In this study, hatchlings also weighed less, had shorter femurs, edema, and were malformed. The toxicity was attributed to the PCB congeners, 105 and 126 and results[193 194] indicated PCB congener 77 as accounting for some of the toxicity in the tern eggs.[192] PCBs have also been implicated as the cause of congenital anomalies and embryonic death in double-crested cormorants (*Phalacrocorax auritus*);[195 196] as possible agents of embryotoxicity in eagles;[197] and in decreased embryonic weight in black-crowned night herons

(*Nycticorax nycticorax*).[198]. Whereas with DDT there is normal embryonic development but death resulting from eggshell thinning with PCBs there is normal shell development but in ovo mortality due to subcutaneous, pericardial, and preitoneal edema and deformities of the beak and limbs.[199] (See dioxin section for more discussion on this topic).

Alligator eggs from Lake Apopka, Florida, have significant residues of PCB's as well as a combination of organochlorine pesticides. [200] Alligators from this site have been documented to have abnormally developed reproductive organs, altered serum hormone concentrations and decreased egg viability. [132, 201, 202] However, alligators from Lake Apopka are known to be exposed to a complex mixture of potential EDCs and therefore it is difficult to pinpoint which compound is responsible for the observed effects. In red-eared slider turtles (*Trachemys scripta elegans*) males, exposed to Aroclor 1242 had significantly lower T concentrations than controls.[203]

The African clawed frog (*Xenopus laevis*) and the European common frog (*Rana temporaria*) were exposed to the PCB mixture Clophen A50 or to PCB 126 for either 10 d or until metamorphosis. Exposed frogs had increased mortality, higher malformation rates, and lower thyroid hormone concentrations.Gutleb, Appelman, et al. 2000 #4639} In a similar study, the same frog species were exposed to the mixtures Clophen A50 and Aroclor 1254 or to PCB 126 and effects of exposure depended on route, time of exposure and length of observations. This study also indicated a relationship between lowered concentrations of retinoid and PCB exposure.[204]. In another study green frogs (*Rana clamitans*) and leopard frog (*Rana pipens*) were exposed throughout metamorphosis to PCB 126 at concentrations ranging from 0.005 to 50 ppb. Survival of larvae decreased at the higher concentrations in both species.[205]

In fish, reproductive impairment has been demonstrated in both in vivo laboratory studies and in field studies of fish residing in PCB-contaminated waters. Several field studies have attempted to correlate PCB tissue levels with observed reproductive alterations. For instance, PCB levels in the liver and ovarian tissue of female English sole from Puget Sound were associated with the spawning of fewer eggs.[206] Similarly, a negative correlation was found between egg hatchability and total PCB concentrations in the eggs of lake trout (*Salvelinus namaycush*) from the Great Lakes.[207] In a study of the reproductive success of lake trout residing in Lake Michigan, Mac and Edsall[208] suggested that maternally derived PCBs were the cause of reduced egg hatchability and increased fry mortality. Johnson et al. [209] reported decreased egg weight and increased oocyte atresia in female winter flounder

(Pseudopleuronectes americanus) with high tissue concentrations of PCBs, although there was no evidence that PCBs altered GSI, plasma E_2, nor fecundity in this species. Interestingly, english sole residing in the same location had reduced plasma E_2 concentrations and impaired gonadal development. It was proposed that the different migratory practices of both fish species might have resulted in different susceptibilities to the chemicals, since these behaviors resulted in differences in the timing and duration of exposure to the most highly contaminated waters.

In the laboratory, female Japanese medaka (*Oryzias latipes*) had reduced GSI and were unable to spawn following an injection of 150 ppm of PCB.[210] It was suggested that PCB exposure disrupted E_2 metabolism, since only control fish, excreted E_2 into the water eight days after the injection. In male goldfish, (*Carassius auratus*) PCB exposure resulted in decreased plasma T and 11KT concentrations, while hepatic EROD activity was increased 15-fold.[211] Reduced plasma T in males and E_2 and P in females, accompanied by an increase in several sex steroid-metabolizing enzymes, was observed in carp (*Cyprinus carpio*) injected with 250 ppm of the commercial PCB Aroclor 1248.[212] In another study, E_2-treated juvenile rainbow trout fed a diet contaminated with PCBs (3, 30, or 300 ppm) for 6 m showed decreased synthesis of VTG.[213].

The decline in estrogens and androgens combined with the elevation of EROD (or other metabolizing enzymes) would suggest that the reduction in sex steroids is related to an increase in metabolism, rather than a decreased synthesis. However, this is probably not the only mechanism for PCB-induced damage, since several studies report abnormalities at the organ level (e.g., GSI, testicular abnormalities) and/or the organism level (offspring survival, hatchability) in fish showing normal sex steroid and VTG concentrations.[214-216] In some of these cases, it is believed that the reproductive abnormalities (e.g., delayed spawning, reduced hatchability) may be caused by the accumulation of toxic levels of PCBs in the ovaries and maturing oocytes.[217] Evidence that PCBs bind VTG suggest that lipoproteins are involved in the transport of the contaminants from extragonadal tissue into the ovaries.[218] One explanation for the inconsistencies observed between studies is that the timing of exposure may be critical to the experimental design. For instance, the lack of effects of TCB on plasma concentrations of sex steroids and vitellogenin in female striped bass (*Morone saxatilis*) and white perch (*Morone americana*) may have been related to the fact that the fish used in these studies were already vitellogenic, and not in a highly active stage of gonadal maturation.[214, 215] The stage of gonadal maturation may also be important in males exposed to PCBs. Atlantic cod (*Gadus morhua*) fed Aroclor 1254 (1-50 ppm) for 5.5 m accumulated significant levels of the PCB in

the testes and liver and expressed considerable testicular damage, including fibrosis of lobule walls, as well as necrosis and disintegration of lobule elements, and decreased spermatogenesis.[219] These authors suggest that the stage of gonadal maturation may be related to the degree of chemical sensitivity, since only males experiencing rapid spermatogenic proliferation or fully mature males suffered testicular damage (i.e. sexually immature and regressed males were unaffected).

In several cases, substantial concentrations of PCBs have been detected in tissues of fish that showed no signs of adverse reproductive effects,[220] It is not surprising that a wide range of responses have been observed in studies that have differed with regards to species and experimental design. However, the chemical complexity of this class of compounds is an additional factor that complicates interpretation. Slight structural differences in the 209 possible PCB congeners, as well as different compositions of the mixtures, may result in vastly different physiological responses.

There is strong evidence that PCBs act at multiple sites along the hypothalamus-pituitary-gonadal (HPG) axis,[141, 221] and in vitro experiments are providing insight into the mechanism(s) underlying these reproductive alterations. However, extrapolating the actual risks that PCBs impose on the environment and biota are difficult due to the complexity and diversity of the commercial mixtures (of congeners). In addition to understanding the interaction of the PBC mixtures with other environmental pollutants and stressors, consideration must be given to the interaction (additivity, synergism, antagonism) of the individual components that make up these mixtures.

PBBs, formed by the bromination of biphenyls, are similar in structure to PCBs. These chemicals are stable and lipophilic and, therefore, present many of the same environmental hazards as PCBs (e.g., persistence in the environment and long biological half-lives). There are 209 possible PBB congeners, although only 45 have been actually synthesized.[222] FireMaster BP-6®, used primarily as a flame-retardant additive in the early 1970s, was the most widely used PBB, although its production was discontinued in 1978.[223, 224] The production and distribution of PBBs was insufficient to result in widespread contamination of the environment, however, the accidental contamination of cattle feed by the Michigan Chemical Company in 1973 resulted in the pollution of many Michigan farmlands. Significant concentrations of PBBs were subsequently detected in water and sediment samples and in tissues of fish and ducks residing downstream of the Michigan Chemical Company.[225]

Although information concerning the reproductive effects of PBBs in fish is lacking, there is substantial

evidence that these chemicals adversely affect reproductive processes in other species.[226] For instance, feeding adult female chickens a diet contaminated with 45 ppm of the commercial PBB FireMaster FF-1 for five weeks resulted in impaired production and hatchability of eggs and was associated with reduced viability of offspring.[227, 228] A variety of reproductive effects following PBB exposure have also been reported in rodents, monkey, cow, mink, and quail.[226 222] Although PBBs have been detected in aquatic environments and are known to accumulate in fish tissues,[229] there is very little information regarding the effects of these chemicals on exposed fish. Like PCBs, PBBs are believed to be potent inducers of several monooxygenase enzymes (including EROD), although it is not known whether this induction affects the metabolism of circulating reproductive hormones.

D. Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzo-*p*-furans (PCDFs)

PCDDs and PCDFs are structurally related compounds produced during a variety of thermal and chemical reactions, including the combustion of PCBs, production of steel and other compounds, and disposal of industrial wastes (via the interaction of chlorophenols). These compounds have also been identified as components of bleached pulp mill effluents. PCDDs and PCDFs are planar chlorinated hydrocarbons with high chemical stability, low water solubility, and limited solubility in many organic solvents. There are 75 possible PCDD congeners and 135 PCDF congeners, although 2,3,7,8-tetrachlorodibenzo-p-dioxin, known as TCDD or dioxin, has received the most attention. Concerns regarding TCDD stem from its wide distribution in the environment and extreme toxicity to both humans and wildlife. Although a variety of PCDDs and PCDFs have been detected in fish and wildlife, the 2,3,7,8-substituted congeners are believed to be the most persistent and prevalent in tissue samples analyzed to date, with half-lives of over a year in some fish species.[230] TCDD and related compounds have been implicated in a number of health related problems, including neurotoxicity, hepatoxicity, cardiotoxicity, chloracne, birth defects, immunosuppression, wasting syndrome, and endocrine and reproductive alterations (reviewed by{Peterson, Theoblad, et al. 1993 #1724}[231, 232]). In non-human primates and rodents, although developmental effects of the immune, reproductive, and nervous systems occur at body burdens in the range of 30-80 ng/kg, biochemical changes on cytokine expression and metabolizing enzymes are seen at doses ten times lower.[231] Many of the toxic effects associated with exposure to dioxins appear to be dependent on target tissue, species, sex, and age.

Studies on the developmental effects of TCDD in rodents have demonstrated that only transient exposure to relatively low concentrations of TCDD during critical windows of development, are capable of eliciting irreversible

disruption of organ functioning in offspring. For example, gestational exposure of rats to low concentrations of TCDD (0.064 –1.0 ppb) during a critical period of development (day 15 of gestation) causes impaired sexual differentiation in male fetuses, including persistence of female traits; decrease in the concentration of T, in the weight of testis and epididymis, and in the production of sperm; and altered sexual behavior during adulthood. [233-235] Similarly, Gray et al., [236] reported a delay in testicular descendent and puberty, with a subsequent reduction in sperm counts and fertility in adult male rodents after a single maternal dose of 1 ug TCDD/kg during day 15 of gestation. Other signs of developmental toxicity in mammals include decreased growth, structural malformations (e.g. cleft palate and hydronephrosis), prenatal mortality, and neurobehavioral changes (e.g. impaired learning in rhesus monkeys).[237]

Laboratory studies on the effects of TCDD in birds have shown significant variation in sensitivity across species, with over 40-fold differences on embryo mortality (reviewed by[237, 238]). For example, doses of only 20 – 50 ppt of TCDD in chicken eggs cause mortality and malformations, as opposed to 1,000 – 10,000 ppt in eggs of ring-neck pheasants (*Phasianus colchinus*) and eastern bluebirds (*Sialia sialis*). Chicken embryos are also much more sensitive to the teratogenic effects of TCDD, particularly to develop cardiovascular malformations.

In birds, there is considerable evidence indicating that embryonic exposure to dioxin and dioxin-like compounds can induce developmental alterations. Indeed, several field studies with colonial fish-eating birds from the Great Lakes, have implicated dioxin equivalents (the aggregate of AhR-active substances) as the causative factors for the increased incidence of developmental deformities and embryo lethality observed in certain contaminated areas (see reviews by[239-242]). All together, these epidemiological studies have provided one of the strongest links between contaminant exposure and reproductive/developmental effects in wildlife. The Great Lakes Embryo Mortality, Edema and Deformities syndrome (GLEMEDS) was first described in double-crested cormorants (*Phalacrocorax auritus*), but has also been reported from other species including the great blue heron (*Ardea herodias*) and the Caspian tern (*Hydroprogne caspia*). The syndrome is characterized by increased embryo mortality; growth retardation; subcutaneous, pericardial, and peritoneal edema; congenital deformities of the bill and limbs; feminization of embryos; and abnormal parental behavior.[239] This syndrome closely resembles the "chick edema disease" observed in chickens after in ovo exposure of hens to PCDDs and PCDFs. Embryotoxicity in fish-eating birds from the Great Lakes has been associated with TCDD concentrations above 100 pg/g (reviewed by [232]). Presently, although a reduction in the release of pollutants to the Great Lakes has resulted in significant population

improvements for several avian species, particularly double-crested-cormorants (*Phalacrocorax auritus*)and ring-billed gulls (*Larus delawarensis*), reproductive and physiological alterations due to contaminants are still associated with population-level effects in birds that feed on highly contaminated fish (such as Caspian terns and bald eagles, *Haliaeetus leucocephalus*).[241, 243] Similar reproductive and developmental effects due to PCDDs have also been reported from free-ranging populations of great blue herons,[244, 245] double-crested cormorants,[246] and wood ducks (Aix sponsa)[247]sampled elsewhere. In addition, in ovo exposure to dioxins has been associated with the development of asymmetric brains in wild (great blue herons (*Ardea herodias*), double-crested cormorants, and bald eagles) and domestic species (chickens, *Gallus gallus*).[248] The behavioral and/or physiological repercussions of this gross brain deformity, however, are unknown at this time. Other sublethal effects observed in birds exposed to dioxins include decrease bursa and spleen weights in developing embryos[249] and altered thyroid gland structure, circulating thyroid hormones, and vitamin A (retinoid) status (reviewed by[250]). In Belgium and the Netherlands, PCDDs/PCDFs in common tern (*Sterna hirundo*) were correlated with lower yolk sac retinoids and plasma thyroid concentrations in hatchlings, and to unfavorable breeding parameters (delayed laying and smaller eggs and chicks).[251] Similarly, cormorants (*Phalacrocorax carbo*) hatchlings from a PCDDs/PCDFs contaminated site in the Netherlands, had decreased plasma thyroid concentrations, and an increased in ovo respiration rate.[252] Results from laboratory studies, however, have failed to replicate what has been reported from wild avian species, and in ovo exposure to TCDD has caused either increases or no changes in thyroid hormones.[253-255]

Information on the developmental toxicity effects of TCDD in amphibians and reptiles is scarce. Neal et al. {Neal, Beatty, et al. 1979 #2202} reported no effects in tadpoles and adult bullfrogs (*Rana catesbeiana*) after a single injection of TCDD (500 ppb). Jung and Walker[256] exposed anuran eggs and tadpoles to TCDD for 24 h and observed that American toads (*Bufo americanus*) treated with at least 0.03 ppb appeared to metamorphose earlier than controls, and that metamorphosis tended to occur at larger body masses after exposure to higher doses of dioxin. The authors concluded that anuran eggs and tadpoles eliminate TCDD more rapidly, and are 100 to 1,000-fold less sensitive to its deleterious developmental effects when compared to fish. Differential sensitivity to TCDD and related compounds could be related to differences in metabolism and/or to different patterns in AhR binding and signal transduction across taxa. In this respect, there is recent information showing a high degree of amino acid sequence conservation for the AhR among bird species (97% amino acid identity), but a much lower percent identity across taxa (79 and 74% identity between the amphibian *Necturus maculosus* and bird and mouse sequences,

respectively).[257]

In a epidemiological study, developmental abnormalities and hatch rates from eggs of the common snapping turtle (*Chelydra serpentina serpentina*) were assessed in relation to over 70 polychlorinated aromatic hydrocarbons, including 8 PCDDs and 14 PCDFs.[258] This study found an increase in the frequency of deformities with increasing contaminant exposure in eggs, particularly PCDDs and PCDFs concentrations. In the laboratory, American alligators eggs (embryo stages 19 –22) were treated with TCDD (at doses ranging from 0.1 to 10 mg/kg), and incubated at male-producing temperatures (33°C).[259] High doses of TCDD in eggs resulted in dose-dependent alterations in sex ratios, with a higher incidence of female hatchlings.

Results from several field and laboratory studies have established that fish, in particular early life stages, are extremely sensitive to the effects caused by TCDD when compared to other taxa. Effects on fry survival are significant at egg doses ranging from 50 to 5000 pg/TCDD/g, which corresponds to concentrations of 75 to 750 pg TCDD/g in parent fish.[232] Signs of TCDD-induced developmental toxicity resemble blue-sac disease, which is an edematous syndrome characterized by yolk sac and pericardial edema, subcutaneous hemorrhages, craniofacial malformations, retarded growth, and death (reviewed by[260]). This syndrome has been well characterized in salmonids after exposure of eggs via water, injection, or through maternally derived TCDD. Studies with salmonids have established differential sensitivity to induce sac fry mortality, with LD_{50} values varying from less than 100 pg TCDD/g egg in lake trout (*Salvelinus namaycush*, the most sensitive fish species to TCDD developmental toxicity), to 200 and over 300 pg TCDD/g egg in brook trout (*Salvelinus fontinalis*) and in some strains of rainbow trout (*Oncorhynchus mykiss*), respectively.[261-263] TCDD-developmental toxicity has also been reported in several non-salmonid species, including the northern pike (*Esox lucius*), the mumichog (*Fundulus heteroclitus*), the Japanese medaka (*Oryzias latipes*), [264] and the zebrafish (*Brachydanio rerio*).[265] Regardless of species or egg exposure route, early life stage mortality occurs during the sac-fry stage, probably as a consequence of the generalized edema.

Exposure of eggs to TCDD and related compounds may have been responsible for the decline of some fish populations in the Great Lakes, since concentrations of dioxin and dioxin equivalent in eggs and fry of salmonids have fallen within the range of those known to induce blue sac disease in the laboratory.[266] The reader is advised to refer to[266] and[267] for a comprehensive review on the effects of TCDD and related compounds on fish of the Great Lakes.

Fish reproduction can also be affected after exposure to TCDD. In the laboratory, adult female zebrafish

fed 5 - 20 ng TCDD showed impaired oocyte development with fewer eggs produced, and lethal developmental abnormalities in offspring (e.g., malformations of notocord).[265] In a separate study, although TCCD treatment of newly fertilized zebrafish eggs did not affect hatchability; doses of 1.5 ng TCDD/g or more resulted in a variety of structural and physiological abnormalities in larvae.[268]

The mechanism(s) by which TCDD and structurally related compounds cause endocrine/developmental effects are complex and not completely understood. The relative toxicity of TCDD and other halogenated aromatics, is likely dependent on their ability to bind and activate the AhR. Although this AhR mechanism is known to be involved in the antiestrogenic action of TCDD, as well as in its ability to induce structural malformations, its mode of action in causing other reproductive and developmental toxicity is less clear.[269] There is substantial evidence that these contaminants induce the expression of certain genes (e.g., Phase I and Phase II enzymes), while altering the transcription of others (ER). Indeed, the antiestrogenic effects of TCDD in mice have been attributed to its ability to suppress ER gene expression probably through an inhibition of ER transcription after binding of the TCDD-AhR complex to promoter regions of the ER gene (see [270] for a review of mechanisms of action of dioxins). Antiestrogenic effects of these contaminants have also been documented in vitro using fish cell lines. Using carp hepatocytes, Smeets et al. [271] demonstrated, that although low concentrations of TCDD caused a suppression of VTG secretion and an induction CYPIA, both phenomena were not correlated to each other. From these results, the authors concluded that the antiestrogenic effects of TCDD were probably not caused by increased metabolism of E_2 due to induction of CYP1A. Mechanistic studies using mammalian cell lines also support this theory,[272] although increased metabolism of sex steroids may provide an additional or secondary mechanism of antiestrogenicity. In this respect, great blue heron hatchlings and adults exposed to TCDD had increased testosterone hydroxylase activity, phenomena that was coupled with increased P4501A1 activity.[273] Changes in hydroxylase activity, however, have not been associated with alterations on circulating sex steroid concentrations in TCDD-exposed herons.[253, 254, 274] The pituitary appears to be a target organ for TCDD, disrupting normal feedback mechanisms between hormones and LH secretion.[275]

Since vitamin A and thyroid hormones are essential for normal differentiation and development of tissues, alterations in their homeostasis might result in malformations and altered growth. In this respect, there is evidence showing that dioxin may interfere with the metabolism and storage of vitamin A (retinoids)[276] and of thyroid hormones[277] through the co-induction of Phase I (P450) and Phase II (uridine diphosphate-glucoroyltransferase (T_4-

UDPGT) enzymes. In addition, hydroxy metabolites of PCDDs and PCDFs can compete for thyroxine on the transthyretin (the thyroxine binding prealbumin) binding site.[278] Finally, recent evidence suggests that the hemodynamic and teratogenic effects observed in fish fry affected by blue sac disease could be due to the ability of TCDD to induce oxidative stress and oxidative DNA damage. In the Japanese medaka, oxidative stress to the vascular endothelium of developing embryos induces programmed cell death or apoptosis.[279] Apoptosis of vascular cells leads to a generalized loss of function and subsequent mortality.

3.4 Organochlorine pesticides and fungicides:

Organochlorine (OC) pesticides comprise a large group of structurally-diverse compounds used to control agricultural pests and vectors of human disease. Many of these compounds, as well as their metabolites, are extremely persistent due to their chemical stability, low water solubility, and high lipophilicity. The exact mode of neurotoxicity is unknown, although OC pesticides are believed to disrupt the balance of sodium and potassium in nerve cells. The ability of these toxic compounds to bioaccumulate in and often harm unintended species has led to the restricted use of most OC pesticides in the United States. Despite a general reduction in use, several field studies have discovered negative correlations between total OC pesticides and endocrine function in fish, indicating that aquatic wildlife is still being exposed to levels capable of altering endocrine and reproductive parameters. For instance, a negative correlation was found between total OC pesticides and E_2 in male, carp in a large-scale field effort to assess the reproductive health of fish in United States streams.[280] Similar results were reported in largemouth bass (*Micropterus salmoides*) collected from a contaminated (with OC pesticides) site in Florida.[281] In the following section, OC pesticides are discussed according to accepted structural classifications, although effects within the same chemical class may differ drastically. Furthermore, Pickering et al.[282] have suggested that pesticide toxicity is species-specific, and a single species may be differentially susceptible to different pesticides. Indeed, the reported effects and mechanisms of action may vary significantly between the various OC pesticides.

Cyclodienes:

The chlorinated cyclodiene pesticides are lipophilic, stable solids with low solubility in water. Although differing from the dichlorodiphenylethanes in their mode of action, they served a similar function in controlling a variety of insect pests. Examples of pesticides in this class include *endrin, dieldrin, chlordane, toxaphene, telodrin,*

isodrin, endosulfan, heptachlor, and aldrin. Consistent with the nature of organochlorine compounds, the cyclodiene pesticides are persistent in soils and sediments with a half-life of 1-14 y in soils following application. Toxaphene is a complex mixture, which can consist of over 300 congeners of chlorinated bornanes and camphenes; therefore, determining the precise environmental fate of this pesticide poses some challenges, since each structurally unique congener possesses an individual set of chemical properties. Although the production of toxaphene was banned in the United States in 1982,[283] a recent survey of lake trout from the Great Lakes reported toxaphene concentrations ranging from 140-3500 ppb wet weight.[284] In a study of juvenile lake trout, the half-life of toxaphene was 63 d, whereas the half-life of toxaphene following injection of adult fish was approximately one year.[285]

The cyclodienes are believed to produce a wide range of toxic responses in wildlife and adverse effects in laboratory animals. For example, rats exposed to endosulfan had alterations to the nervous, immune, hepatic, renal, and reproductive systems.[286]

Dieldrin levels of 9.4 ppm in purple gallinule (*Porghyrula martinica*) and of 17.5 ppm in the common gallinule (*Gallinula chloropus*) showed no significant effects on percentage of eggs hatched nor in the survival of young.[287] Lockie et al.[288] reported that the proportion of successful eyries of the golden eagle (*Aquila chrysaetos*) increased from 31% to 69% as the levels of dieldrin fell from a mean of 0.86 ppm to 0.34 ppm. Screech owls (*Otus asio*) with egg aldrin concentrations in the egg ranging between 0.12 - 0.46 ppm were 57% as productive as controls, with lower clutch sizes, hatch rates, and survival.[289] Heptachlor epoxide reduced nest success in Canada geese (*Branta canadensis*) when eggs contained > 10.0 ppm[290] and reduced productivity in American kestrels (*Falco sparverius*) when eggs contained > 1.5 ppm.[291] No relationship was found between heptachlor epoxide residues and shell thickness in eggs of Swainson's hawk (*Buteo swainsoni*) nor was reproduction affected in wild prairie falcons (*Falco mexicanus*) and merlins (*Falco columbarius*).[291, 292] Chlordane fed to northern bobwhites (*Colinus virginianus*) in concentrations of 3 and 15 ppm and to mallards (*Anas platyrhynchos*) at 8 ppm, had no effect on reproduction.[293] Similarly, toxaphene fed at 100 ppm to chickens had no significant effect on reproduction.[294] A two year study with American black ducks (*Anas rubripes*) fed a diet containing 1, 10, or 50 ppm toxaphene produced no reproductive effects, although duckling growth, backbone development, and collagen was decreased in offspring of parents fed 50 ppm.[295]

Chlordane, dieldrin, and toxaphene were tested for their ability to override male-producing incubation

temperature in the red-eared slider turtle (*Trachemys scripta elegans*). Chlordane produced significant sex reversal alone and when administered with E_2.[296] In another study, treated male turtles exposed to chlordane had significantly lower T concentrations and females had significantly lower P,T, and 5 α-DHT concentrations than controls.[203]

Studies involving the effects of cyclodienes on the reproductive success of fish have produced a range of results, most likely resulting from species- and chemical-specific sensitivities, as well as differences in experimental design. For instance, toxaphene at concentrations ranging from 0.02 – 2.2 ppt did not affect the reproductive success of female zebrafish, as measured by total number of eggs spawned, percentage of fertilized eggs, embryo mortality, and egg hatchability.[297] In this species oviposition, however, appeared to be affected by toxaphene exposure in a dose-dependent manner. Conversely, decreased fertilization has been observed in winter flounder after exposure to 0.001-0.002 ppm dieldrin,[298]{Smith & Cole 1973 #5069}; reproduction of first-generation flagfish (*Jordanella floridae*) was affected after exposure to 0.3 ug/L endrin;[299] and sublethal concentrations of dieldrin and aldrin were reported to induced abortion in mosquitofish (*Gambusia affinis*).

Although less information is available regarding the effects of cyclodienes on reproductive function in male fish, studies involving *Tilapia rendalli* and a laboratory study[300] on *Oreochromis mossambicus* showed disrupted nest-building, and decreased reproductive activity. In a study of male striped snakehead (*Channa striatus*), testicular damage and disrupted spermatogenesis were observed after exposure to 0.75 - 1 ppm of endosulfan for 2 - 30 d. [301]

Several reports indicate that oocyte development may be a target for cyclodiene-mediated reproductive toxicity. An increase in oocyte atresia was observed in rosy barb exposed to a low dose (46.6 pptr) of aldrin for 2-4 m.[302] Impaired oocyte development and reduced GSI have also been observed in striped snakehead[303] and carp minnow (*Rasbora daniconius*)[304] exposed to endosulfan . Other toxic effects related to endosulfan exposure include reduction in the percentage of maturing and mature oocytes; rupturing of ooctye walls; damage to yolk vesicles; and multiple histopathological changes in ovarian morphology.[303] Consistent with the observations of oocyte damage and decreased GSI, endosulfan was shown to have an inhibitory effect on vitellogenesis in clarias catfish.[305] It is possible that endosulfan directly interferes with vtg synthesis in the liver, a theory that is supported by evidence that endosulfan alters protein synthesis in the liver of *Cirrhinus mrigala* and Clarias catfish (*Clarias*

batrachus). Alternatively, other studies suggest that endosulfan impairs steroidogenesis by interfering with enzymes along the steroid biosynthetic pathway[306] Likewise, the authors of the later study concluded that endosulfan affected VTG synthesis by interfering with the production or activity of hormones responsible for regulating VTG production. Multiple effects along the hypothalamo-hypophysial-ovarian axis of *Sarotherodon mossambicus* were also observed following an exposure to 0.001 ppm endosulfan for 20 d.[307] In addition to reduced GSI and various histopathological abnormalities associated with ovarian growth and oocyte maturation, degeneration of basophiles and acidophils (gonadotrops) in pituitary tissue of endosulfan-treated fish was apparent. Exposures of fish to endrin, another member of the cyclodiene family, may also result in morphological abnormalities of the pituitary and ovaries due to the inhibition of neurohormones at the level of the hypothalamus.

Chlordecones (Kepone and mirex):

Chlordecone, also known as Kepone, and mirex are two structurally similar organochlorine insecticides that were manufactured and used primarily in the 1960s and 1970s. No longer permitted in the United States, mirex was used as a pesticide to control fire ants as well as a flame retardant additive, and chlordecone was used to control insects on a variety of crops and for household purposes.[308, 309] The toxicological effects of chlordecone exposure in humans are well-documented as a result of an incident known as the "Kepone Episode," in which many employees and residents in the vicinity of several Kepone manufacturing companies were exposed to intoxicating concentrations of the chemical.[310] The central nervous system, liver, and reproductive organs appeared to be most sensitive to the toxic effects of chlordecone. Comparative studies using laboratory animals have since concluded that the target organs as well as the excretion pathways for chlordecone are similar in humans and rodents, although metabolic pathways differ significantly.

Reproductive impairment in a variety of mammalian and nonmammalian species has been attributed to the estrogenic properties of chlordecone (reviewed by[310] and[311]. Chlordecone induced constant estrus in mice[312, 313] and neonatal injections in female rodents accelerate vaginal opening and the onset of prolonged vaginal cornification with reductions in ovarian weight.[314] In quail kepone caused oviduct hypertrophy in females[315] and suppressed spermatogenesis in males.[316, 317] Mirex fed to mallards at concentrations of 100 ppm decreased duckling survival and hatch rates were reduced in chickens fed 600 ppm mirex.[318] Chlordecone also reduced hatchability in chicks

when the adults are fed 150 ppm. Survival of chicks from hens receiving 75 ppm of chlordecone was also reduced.[319]

In fish, there is evidence that chlordecone competes with radiolabeled E_2 for binding to the hepatic ER in spotted seatrout (*Cynoscion nebulosus*),[143, 320] rainbow trout,[321] Atlantic croaker,[322] and channel catfish. [323] Several in vivo studies report the induction of VTG following exposure to chlordecone and mirex, which suggests that these chemicals exert their effects by acting as estrogen-mimics. While many investigators have observed VTG stimulation in response to chlordecone exposure, others report a suppressive effect or no effect at all by chlordecone or the related pesticide, mirex.

Other alterations attributed to chlordecone exposure include inhibition of oviposition in *Oryzias latipes;*[324] reduced egg production and decreased hatchability in *Cyprinodon variegatus;* and histopathological abnormalities in freshwater catfish (*Heteropneustes fossilis*). For instance, exposure of female catfish to chlordecone (0.024 ppm) for 1-2 m resulted in a decrease in the diameter of stage 1-3 oocytes, the formation of interfollicular spaces in the ovaries, and an increase in oocyte atresia.[325] Male catfish in the same study also had a variety of histological testicular abnormalities. Sub-acute doses (0.024 ppm) over the same time period resulted in significant damage to the seminiferous tubules and cystolysis of spermatids and sperm.

Dichlorodiphenylethanes:

The dichlorodiphenylethane pesticide reported most often as having endocrine activity is 1,1,1-trichloro-2,2-Bis *p*-chlorophenylethane (DDT). Used extensively during World War II to control insect borne diseases DDT was released into the environment in substantial quantities and, consequently, accumulated in soil, water, and tissues of many animals, including fish. The *p,p'*- and *o,p'*-substituted isoforms of DDT; the dechlorinated analogs, *p,p'*- and *o,p'*-DDD; and the metabolites *o,p'*- and *p,p'*-DDE are various forms that frequently exist in the environment. In highly polluted areas (e.g., Palos Verdes Shelf in Southern California), concentrations of ΣDDT (total measured DDT, DDE and DDD) have exceeded 100 ppm wet weight in the livers of several species. *p,p'*-DDE is one of the most commonly detected and highly persistent OCs in tissues of aquatic animals, and in a recent study by the USEPA (1991) this metabolite was detected in 98% of fish surveyed at 388 locations in the United States. Over the last two decades, concentrations of DDT and its derivatives have decreased in fish of the United

States, Canada, western Europe, and Japan as a result of strict regulation on its use. In addition to DDT, this category of OC pesticides includes DMC (Dimite), dicofol (Kelthane), methlochlor, methoxychlor, and chlorbenzylate.

Laboratory tests have shown increased uterine weight and persistent vaginal estrus in rats exposed to *o,p'*-DDT.[326, 327] Mammals concentrate DDT in adipose tissue where it can become toxic when fat is lost due to migration or hibernation and the pesticide is unbound. [328] Although concentrations in wildlife have decreased since DDT was banned, little information exists linking DDT exposure in the environment to estrogenic or adverse reproductive effects in wild mammals.[311]

The introduction of OC pesticides, especially DDT, was responsible for the declines in populations of many species of predatory birds during the 1950s and 1960s. Bird declines were due to eggshell thinning with DDE being responsible for most of this problem, however, some species are more sensitive than others. For example, 3.0 ppm DDE in egg produces eggshell thinning and reduced productivity in the brown pelican (*Pelecánus occidentális*) with residues > 3.7 ppm leading to total reproductive failure. On the other hand, peregrine falcons are less sensitive, and reproductive depression first occurs at egg residues of 30 ppm. Black-crowned night herons (*Nyctícorax nyctícorax*) have a gradual decline in productivity as residues increase. Eggshell thinning due to DDE has also affected populations of bald eagles (*Haliaeetus leucocephalus*).[329] The mechanism for eggshell thinning although previously thought to be caused by the estrogenic effects of DDE is now proposed to involve the inhibition of prostaglandin synthesis in the eggshell gland muscosa.[329, 330] Additional reports of DDT effects in birds include decreased egg hatchability in eastern bluebirds (*Sialia sialis*).[331]

Eggs from alligators from Lake Apopka in Florida are know to contain residues of OC pesticides including toxaphene, dieldrin, DDE, DDD, and chlordane, as well as PCBs.[332] Several studies have described adverse reproductive effects to the alligator population on this lake including increased embryo mortality,[333, 334]and morphological and endocrine abnormalities in juvenile alligators. [131, 335]. DDE also has been shown to cause sex reversal in alligators and red-eared slider turtles (*Trachemys scripta elegans*) following the treatment of eggs at early embryonic stages and at incubation temperatures necessary for the production of male offspring.[296] DDT has also been reported to induce VTG in the red-eared slider as well as in frogs (*Xenopus laevis*).[336] Clark et al.[337] reported that technical-grade DDT acted as an antiestrogen and *p, p'*-DDE as an estrogen in

Ambystoma tigrinum (tiger salamander). In a study on the reed frog *Hyperolius argus*: *o,p* DDT, *o.p'* DDE and *o, p'* DDD prematurely induced adult female color patterns in juveniles however this was not repeated by p,p'DDT, p,p' DDE, and p,p' DDD.

A number of studies have demonstrated the effects of DDT and related compounds on the reproductive success of exposed fish. Increased fry mortality has been observed in Brown trout (*Salmo trutta*) and brook trout exposed to DDT concentrations ranging from 0.5 - 3.4 ppm/week for 98-308 d.[338] Fry mortality was also reported in a field study of lake trout (*Salvelinus namaycush)* inhabiting a lake polluted with DDT.[339]. In the laboratory, white croaker collected from a contaminated site were unable to spawn when total ovarian DDT concentrations exceeded 4 ppm. An increase in oocyte atresia and reduced fecundity and fertility were also observed in white croaker from a DDT contaminated site.[340] Organism and population level effects due to DDT and derivation include decreased fertilization and embryo deformity in winter flounder;[341] decreased fertility and early oocyte loss in *Genyonemus lineatus;*[342] and egg mortality in artic char (*Salvelinus alpinus).*[343] Functional male-to-female sex reversal in Japanese medaka has also been reported following the injection of approximately 227 ng *o,p'* DDT/egg during the course of fertilization. A reduction in viable hatch has also been observed in *Clupea harengus* chronically exposed to 0.018 mg DDE/kg ovary.

Substantial evidence from in vitro studies (particularly in mammals) suggests that DDT and related OC pesticides are estrogenic and, thus, mediate endocrine disruption through interaction with the ER. Several in vivo studies in fish have tested this theory by evaluating the effects of DDT and related compounds on E_2-dependent processes, such as vitellogenesis. A study by Spies et al.[344] found that female kelp bass from a polluted site had lower GTH, T, and E_2 concentrations compared to fish from a reference site. Furthermore, the rate of GTH release from the pituitary was enhanced and correlated with hepatic concentrations of DDT. In laboratory studies, an increased rate of GTH release from the pituitary of female kelp bass exposed to DDT was consistent with field observations, yet T production in laboratory exposed fish treated in the laboratory was enhanced. Receptor binding studies found that E_2 binding to the ER was reduced in DDT-exposed fish and conversely DDT was capable of displacing E_2 from the ER. While the estrogenic potential of DDT may vary among different species of fish and other vertebrates, the above studies demonstrate the complexities of the reproductive system and the difficulty when interpreting alterations induced by this group of OC pesticides.

Hexachlorocyclohexane:

Hexachlorocyclohexane (HCH) is an organochlorine chemical for which there are eight isoforms and several are used to prepare the technical-grade product. HCH was primarily used as an agricultural pesticide and is, to some extent, still applied as an insecticidal seed dressing. The precise mode of toxicity is unclear, although the a- and l- isomers are known convulsants, and the b- and d-isomers are CNS depressants. The refined product, known as g-HCH, g-BHC, or lindane, consists primarily of the g-form. This product has received extensive use as an insecticide for fruit, vegetable, and forest crops, and as a component of the ointments used to treat head and body lice. Lindane shares many chemical properties with other organochlorine pesticides, although it has greater polarity and water solubility than most. Although in the United States its production was discontinued in 1977, lindane is still imported and used by EPA certified applicators. Lindane can accumulate in the fatty tissues of fish; however, it is easily degraded to less toxic metabolites by algae, fungi, and bacteria inhabiting soil, sediment, and water.[345] Less water-soluble than its gamma counterpart, b-hexachlorocyclohexane (b-HCH; a byproduct generated during the synthesis of g-HCH) is the most persistent of the HCH isomers and has been known to bioconcentrate in the tissues of invertebrates, fish, birds, and humans.

Wester et al.[346-348] have examined the effects of b-HCH on the development of the reproductive organs of several fish species. Four-week old guppies and post-fertilization Japanese medaka eggs were exposed to a range of concentrations (0.0032-1.0 ppm) for 1-3 m,[348, 349] Female guppies exposed to 0.32-1.0 ppm had a high incidence of premature, and abnormal yolk formation despite having no fully mature oocytes. In the absence of developed oocytes, VTG accumulated in the body fluids, including the glomerular filtrate of the kidneys, and may have contributed to several toxic lesions observed in various non-reproductive tissues. VTG was also detected in male guppies exposed to 0.32 and 1.0 ppm b-HCH, and GTH production by the pituitary was activated, although, testicular development was delayed and evidence of intersexuality or hermaphroditism was reported. Japanese medaka exposed to b-HCH also exhibited intersexuality or hermaphroditism following exposure to concentrations higher than 0.1 ppm and treatment with several estrogenic substances has previously been shown to induce the development of a testis-ova in this species. Overall, these authors concluded that the alterations observed in both species were the direct result of the estrogenic activity of b-HCH and/or its metabolites. Singh and colleagues[350-352]

conducted a series of in vivo studies that examined the effects of g-HCH (lindane) on steroidogenesis at different phases of the reproductive cycle of goldfish (*Carassius auratus*) and two species of catfish (*Clarias batrachus* and *Heteropneustes fossilis*). Regardless of reproductive stage, four weeks of exposure to 8 ppm g-HCH resulted in reduced plasma T and E_2 concentrations in female clarias catfish, although effects at this dose were more dramatic in females in the later stages of vitellogenesis.[352] Similar results were obtained following exposure of female freshwater catfish to 4 and 8 ppm g-HCH for four week. [350] A decrease in T and E_2 was observed in all stages of females examined (preparatory, pre-spawning, spawning, post-spawning, and resting), and sensitivities appeared to increase from the preparatory to the spawning phase. Although the exact mechanism underlying the effects of g-HCH remains unknown, it has been suggested that this insecticide may inhibit gonadal recrudescence by reducing GTH secretion or the number of GTH receptors, which would likely interfere with steroidogenesis.[352, 353]

Vinclozolin:

Vinclozolin (3-(3,5-dichlorophenyl)-5-methyl-5vinyl-oxazolindine-2.4-dione) is a dicarboximide fungicide is used on vegetables and fruits. Two metabolites, M1 and M2, have been reported to be anti-androgens. Pregnant rats that received vinclozolin during gestation produced male offspring with reduced anogenital distance, cleft phallus, retained nipples, and hypospadias.[354, 355] Laboratory studies showed Leydig cell hyperplasia, testicular tubular cell atrophy, penile hypoplasia, as well as hypospadia and infertility in male offspring.[356] Vinclozolin is an antiandrogen exerting its effects by binding to the androgen receptor.[311, 357] However, effects in wildlife have been poorly characterized and environmental use is limited.

3.5 Non-organochlorinated pesticides:

Organophosphate pesticides (OPs):

Organophosphate chemicals have been used as nerve gas and insecticides. Their effectiveness as chemical warfare agents and insecticides stems from their ability to inhibit acetylcholinesterase, an enzyme required for basic nerve conduction. The majority of OPs are lipophilic liquids, although they tend to have greater polarity and water-solubility than OC pesticides. Due to their innate instability, most OP pesticides are not believed to bioconcentrate in aquatic species, although select fish species are highly sensitive to the toxic effects of these chemicals. Currently,

many OPs are still used in a number of countries to protect crops from insects, and farm and domestic animals from endo and ectoparasites. They may also be used to control product pests disease vectors (e.g. mosquitoes).

There is some indication showing that OPs might induce endocrine-disrupting effects in wild mammals. Reproductive depression, including decreased percentage of females with embryos and a decreased percentage of births, has been observed in rodents (*Sigmodon hispidus*, *Microtus ochrogaster*, and *Reithrodontomys fulvescens*) exposed to diazinon.[358] Reproductive activity was also depressed in *Sigmodon hispidus* exposed to carbaryl.[359] A recent study on the population effects of terbufos, however, found no changes on reproductive activity, number of births, or litter size in deer mice (*Peromyscus maniculatus*) and white-footed mice (*P. leucopus*).[360]

In birds, there is some evidence showing altered gonadotrophin release after exposure to OPs. Japanese quail (*Coturnix coturnix japonica*) exposed to 10 mg/kg parathion responded with a decrease in LH levels.[361] Since this effect was observed at near lethal concentrations (brain cholinesterase activity was inhibited by over 50%), it is unclear whether this represents a case of endocrine disruption or acute toxicity. Other laboratory studies have reported changes on incubation behavior and egg laying,[362-365] and decreased gametogenic function in adult birds exposed to OPs.[366] OPs are also known to induce developmental toxicity effects in birds (see review by [367]), such as short limbs and parrot beak (known as Type I defects) and skeletal deformities (Type II defects). Results from field studies, however, have generally failed to find effects on several reproductive success parameters (clutch size, hatchability, and number of young fledged/nest) in populations of birds exposed to OPs after agricultural spraying.[368-371]

Exposure of amphibians to OPs may result in altered metamorphosis. Development of bullfrog (*Rana catesbeiana*) tadpoles was significantly delayed after exposures to at least 1,000-ppb malathion, possibly because of decreased thyroid function.[372] These concentrations, however, are above those commonly found in wetlands or streams after pesticide application. In a separate study, exposure of premetamorphic northern leopard (*Rana pipiens*) and green frogs (*R. clamitans*) to environmentally relevant concentrations of OPs (< 0.01 ppm of basudin 500EC and technical grade diazinon) caused deformities and delayed development.[373] Although exposure to the OP methyl parathion (1 mg/L) has been associated with bone deformities in amphibians, similar to the Type II defects observed n birds,[374] its effects on metamorphosis are less clear.[375]

Chronic exposure to low environmental concentrations of OP pesticides may lead to a variety of

reproductive and developmental effects in fish. For example, Ram and Sathyanesan[376] exposed murrel (*Channa punctatus*) to 20 mg/ml cythion (50% malathion, 50% organic solvents) for 6 months and observed an increase in oocyte degeneration, which resulted in retarded ovarian growth and lower GSI. These responses were correlated with fewer and less active gonadotropin-producing cells in the pituitary. In males, spermatogenesis was arrested and some necrotic spermatocytes were apparent. The authors speculated that reduced GTH levels might have contributed to the observed reproductive abnormalities. In freshwater perch (*Anabas testudineus*), although no short-term effects were observed after an exposure to 0.106 ppb Metacid-50 (50% methyl parathion), a reduction in GSI and plasma and ovarian E_2 concentrations was evident after 20 days.[377] Exposure to OP pesticides has been documented to elicit a series of histological alterations in ovaries of several fish species. Exposure to 0.1 mg/L methyl parathion for 75 days resulted in substantial oocyte damage in carp minnow (*Rasbora daniconius*).[304] Similar effects have been observed in guppies *(Puntius conchonius*) exposed to 53 ug/L monocrotophos for 2-4 months.[378] Guppies exposed to fenitrothion have also responded with decreased egg production and abortion.[379] Gonad weights and vitellogenesis were reduced in female striped catfish (*Mystus vittatus*) after 12 weeks of exposure to four different organophosphate pesticides (malathion, birlane, gardona, and phosdrin).[380] Histological abnormalities of the testis has been a common response in male fish exposed to OP pesticides, having been reported in at least 10 fish species.[381-391] Recent evidence indicates that exposure of newly hatched larvae to malathion might also result in developmental alterations (deformed notochord).[392]

Exposure of fish to OPs has also been associated with declines in the concentrations of hormones and VTG. For instance, exposure to malathion and to cythion results in reduced plasma concentrations of E_2, T, and VTG. [388] In another study, female catfish (*Heteropneustes fossilis*) had decreased plasma E_2 concentrations after an exposure to 1.2 mg/L malathion for 72 h.[393] Similar results were reported in studies examining the effect of malathion on sex steroid concentrations during different phases of the reproductive cycle of the catfish (*Clarias batrachus*).[394] It was concluded from this study that, sensitivity to malathion appeared to increase from the pre to the post-vitellogenic phases, the latter of which involves ovulation and spawning. Carbaryl-induced thyroid dysfunction has also been reported in this species of freshwater fish.[395]

Evidence suggests that OP pesticides may affect steroidogenesis by acting at multiple sites along the hypothalamus-pituitary-gonadal-liver axis. In fish, exposure to certain OPs reduces GnRH-like factor levels in the

hypothalamus, and impairs pituitary activity and release of GTH.[396-398] Additional studies have shown reduced 3-HSD and 17 -HSD activities in ovaries and testis of exposed fish.[385, 399] Singh[387] proposed that malathion reduces E_2 in the Asian rice eel, *Monopterus albus*, by interfering with the enzyme aromatase. Malathion, however, appears not to affect cholesterol biosynthesis, although it has been shown to alter the synthesis and mobilization of other lipids, as well as the hydrolysis of esterified cholesterol to free cholesterol.[400, 401]

Carbamate pesticides: The carbamate pesticides, many of which are in current use, are derivatives of carbamic acid. Like the OP pesticides, they act as acetylcholinesterase inhibitors, vary with regard to water solubility, and are relatively nonpersistent in the environment. Carbaryl is a carbamate pesticide that controls over 100 species of insects on a variety of crops, agricultural animals, and pets. Although carbaryl has been reported to accumulate in certain species of fish and invertebrates, the risk of biomagnification is low due to its rapid metabolism and degradation. Carbofuran is another carbamate pesticide with wide application, although granular forms were banned in the U.S. in 1994 following a number of bird kills. Carbofuran is used to protect field, fruit, vegetable, and forest crops from insects, mites, and nematodes.

Laboratory studies with amphibians, have reported growth inhibition and increased incidence of developmental deformities in tadpoles exposed to carbamate pesticides.[373, 374, 402] In fish, carbamate pesticides have been shown to induce histopathological alterations in the ovaries and testes. Female fish exposed to carbofuran (range of 1 to 5 ppt) have responded with reductions in GSI, inhibitions in oocyte growth, and increases in oocyte atresia.[381, 403] Some of the lesions seen in ovarian tissue of carbofuran-exposed fish have included decreased oocyte diameter, a predominance of immature oocytes, and damage to yolk vesicles and oocyte structure.[404, 405] In males, carbofuran causes declines in testes weight, delays in spermatogenesis, and induces necrosis of spermatogonia and spermatocytes.[381, 382] Although generally less toxic to fish than carbofuran, carbaryl exposure results in many of the same reproductive alterations. Carbaryl-induced alterations, including reduced GSI and plasma E_2 concentrations, inhibited oocyte growth, increased oocyte atresia, and damage to yolk vesicles and oocyte structure, have been observed in several species of fish after exposures ranging from 2 to 20 mg/mL.[377, 406 407] Increased larval mortality and decreased production and hatchability of eggs are additional responses to carbaryl exposure.[408]

Little information is known regarding the mechanism(s) by which carbamate pesticides induce reproductive anomalies in fish, although results from both field and laboratory experiments suggest that carbaryl may act at the

level of the pituitary by altering GnRH and GTH serum concentrations.[396] Results from the in vitro ESCREEN indicate that neither carbaryl nor carbofuran are estrogenic in nature.[409] Carbofuran also appears to raise cholesterol and phospholipid concentrations in the ovaries and testes of fish, while lowering overall protein, RNA, total lipids, and ascorbic acid. [410]

Organometal pesticides: Certain metals are highly insoluble in their inorganic form and, therefore, possess little to no toxicity. However, since metal toxicity may be greatly enhanced if binding to an organic ligand occurs, some metals have been modified intentionally to increase their toxicity for use as pesticides. Until 1993, organomercury was used as an antifungal seed dressing in the UK; organolead has been applied to fruit crops to control caterpillars; and organotin compounds have served a number of functions due to their extreme toxicity. For example, tributyltin (TBT) has served as an algicide, miticide, fungicide, and insecticide, and since the 1960s, it has also functioned as a marine antifouling agent. Both agricultural and maritime applications have lead to the contamination of aquatic environments. Although the half-life of TBT in water is brief (days to weeks), organotin compounds have the potential to bioaccumulate in aquatic organisms.

One of the best documented cases of endocrine disruption comes from the work done with marine gastropods exposed to organotin compounds (mainly TBT) contained in antifouling paints. Laboratory and field studies have demonstrated that female gastropods exposed to environmentally relevant doses of TBT develop an irreversible sexual abnormality known as "imposex". This masculinization process involves an increase in T concentrations, which is followed by the imposition of male sex organs (penis and vas deferens) over the oviductal tissues, causing abnormal breeding activity and in many cases, population declines.[411-416] Depending on the species and dose attained, oogenesis might be completely supplanted by spermatogenesis. TBT can also induce alterations in the behavior and development of bivalve larvae.[416] It is estimated that about 72 species and 49 genera of prosobranchs are affected world-wide (see review by [417]). In the case of the highly sensitive common dogwhelk (*Nucella lapillus*), imposex is induced at exposures as low as 1 – 2 ng/L, with complete suppression of oogenesis at TBT concentrations above 3 – 5 ng/L .[412]

In fish, organotin compounds are readily bioaccumulated and stored in different tissues, including the gonads.[418] Exposure to organotins has been associated with delayed hatching, high embryo and larval mortality, and retarded yolk sac resorption in several fish species.[419 420 421 422-424] In guppies, exposure to TBT (11.2 – 22.3 ng/L)

and bisphenol A (274-549 ug/L) results in significant declines (by 40 – 75%) in total sperm counts after 21 days.[425] Organotin compounds also induce several alterations in ovaries and testes of fish. Three-spined sticklebacks, *Gasterosteus aculeatus*, exposed to bis(tributyltin)oxide (TBTO) for up to 7.5 months experienced no seasonal increase in GSI, as was apparent in control animals. In addition, ovaries from exposed animals contained 25% percent resorbing oocytes, as opposed to 0% in controls.[426] In this study, however, several other reproductive endpoints (including spawning behavior, fecundity, hatchability, frequency of deformed fry, and secondary sex characteristics) were not affected by treatment.

Although the precise mechanism(s) by which TBT causes endocrine disruption in invertebrates are not entirely known, recent evidence suggests that this compound may act as a competitive inhibitor of cytochrome P450-mediated aromatase.[411, 413] TBT may also interfere with sex steroid metabolism, inhibiting the formation of sulphur conjugates of T and its active metabolites. In addition, TBT is capable of inducing cytotoxic and genotoxic damage to embryonic and larval stages in invertebrates.[427, 428]

Triazine pesticides: The triazine pesticides include some of the most extensively used herbicides in North America. Indeed, atrazine is used to control weeds on more than two-thirds of the U.S acreage containing corn and sorghum, as well as 90% of sugarcane acreage. Simazine, another member of this class of herbicides, is currently applied to 30 high-value crops including a variety of fruits, vegetables, nuts, turfgrass, and conifers. Despite the widespread use of these chemicals, relatively little is known regarding their potential health effects to humans and wildlife. Atrazine is only slightly toxic to fish and the risk for bioaccumulation is extremely low due to its propensity for rapid degradation to less- or non-toxic metabolites.

The effects of atrazine on amphibian metamorphosis have been examined in some detail. Tiger salamanders (*Ambystoma tigrinum*) exposed to low concentrations of atrazine (75 ug/L) developed at slower rates, but were similar in size when compared to controls.[429] In contrast, exposure to high atrazine concentrations (250 ug/L) resulted in similar developmental rates, but decreased sizes. In addition, plasma thyroxine (T_4) was elevated in both groups, whereas corticosterone was depressed in the low-dose group only. These authors hypothesized that the suppression of corticosterone could have resulted in a decreased conversion of T_4 to the active form 3,3', 5-triiodothyronine (T_3), thus slowing metamorphosis and allowing increased growth. In contrast, Allran and Karasov[430] found no effects on developmental rate and metamorphosis in northern leopard frogs (*Rana pipiens*) exposed to atrazine (20 and 200 ug/L). Atrazine has also been shown to induce teratogenic changes in frog

embryos, but at concentrations approaching maximum solubility in water.[431]

Studies with alligators have shown that atrazine might induce differential responses in developing embryos depending on timing of exposure. Crain et al.[432] reported that atrazine (14 mg/L) induced gonadal aromatase activity in male hatchling alligators exposed in ovo. In a later study, however, incubation of alligator eggs with atrazine prior to the critical period of gonadal differentiation, did not influence sex determination, and had no apparent effect on gonadal structure (measured as sex-cord diameter in males, Mullerian duct epithelial cell height, and medullary regression of the ovaries in females), nor hepatic aromatase activity.[433] Since most endocrine changes associated with atrazine have been reported in normally organized reproductive systems, the authors hypothesized that the lack of noticeable effects in the latter study was the result of exposing embryos during very early developmental stages, i.e. prior to or during the development of the reproductive system.

Few studies have examined endocrine or reproductive function in fish exposed to atrazine or other triazine pesticides. Channel catfish (*Ictalurus punctatus*) and gizzard shad (*Dorosoma cepedianum*) maintained for 4.5 months in ponds containing 20 ug/L atrazine failed to reproduce, and reproductive success of bluegills (*Lepomis macrochirus*) was reduced more than 95%.[434] Since the dietary habits of bluegill were largely affected by the herbicide treatment, the authors suggested that impaired reproduction might have been due to impoverishment rather than to direct effect of atrazine exposure. Results from our laboratories have shown that atrazine affects sex steroids in male and female largemouth bass (*Micropterus salmoides*) (Gross et al., unpublished data). After 20 days of exposure, plasma 11-KT concentrations were elevated in males exposed to 100 ppm atrazine, and E_2 concentrations were increased in females exposed to 50 and 100 ppm atrazine. Studies with largemouth bass have also shown that when ovarian follicles are incubated with 10 ppm atrazine, it results in an increased E_2 and a decreased T production. Furthermore, in vitro T synthesis is greatly reduced when gonads are incubated with a combination of atrazine and floridone or atrazine and chlordane.

Current evidence suggests that atrazine induces endocrine-disruptive effects by acting as an steroid hormone antagonist (antiandrogen and/or antiestrogen), probably through non-receptor mediated mechanisms. Indeed, a number of in vivo and in vitro studies have failed to detect estrogenic activity for triazines. In two independent studies, oral exposure to atrazine and simazine did not increase uterine weight in immature or ovariectomized female Sprague-Dawley rats,[435, 436] and cell proliferation and binding studies found no evidence for either agonistic or antagonist activity for this herbicide.[435] Furthermore, atrazine and related compounds failed to

demonstrate estrogenic activity in human and yeast cells expressing the ER and an estrogen-sensitive reporter gene,[437, 438] although the triazines have displaced radiolabeled estradiol from the ER in competition studies.[363, 438] Also, a study by Danzo,[439] showed that atrazine did not reduce radiolabeled estradiol binding to rabbit uterine ER, although it inhibited the binding of dihydrotestosterone to androgen receptor sites in rat testes and reduced the binding of dihydrotestosterone to the androgen binding protein by 40%. Triazines may also disrupt reproductive function by altering LH and prolactin concentrations.[440]

3.6 *Complex Mixtures*:

Pulp and Paper Mill Effluents

Over the past 15 years, a number of investigators have studied the effects of pulp and paper mill effluents on feral and laboratory fish populations. In general, fish exposed to these effluents experience alterations in steroid biosynthesis, gonadal development, sexual maturation, and expression of secondary sex characteristics. Identifying the causative agents in the effluent and establishing cause-and-effect relationships, however, have been challenging tasks, since pulp and paper mill effluents are complex mixtures, and the components are not entirely known. Furthermore, variations in wood finish; in the pulping and bleaching process; and in the treatment of effluents between mills leads to different effluent compositions. Nevertheless, all pulping protocols involve the separation and discharge of natural wood components such as sugars, lipids, resins, and fatty acids, which generally undergo bacteriological treatment in settling and aeration ponds. Depending on the bleaching techniques used, pulp and paper mill effluents may also contain different kinds and concentrations of chlorinated organic compounds such as PCDDs and PCDFs.

The most thorough field studies on the reproductive effects of paper mill effluents have been conducted at Jackfish Bay, Lake Superior. Jackfish Bay, has received bleached kraft mill effluent (BKME) from a nearby pulp mill since 1949 and, therefore, has provided a convenient site for studying the impact of BKME on several fish species. BKME-exposed white suckers (*Catostomus commersoni*) show decreased concentrations of several sex steroid hormones (T, 11-KT, and E_2).[441-445, 445-447] Declines in steroid concentrations have also been documented in longnose sucker (*Catostomus catostomus*) and lake whitefish (*Coregonus clupeaformis*) from Jackfish Bay;[442, 442, 444] in white sucker at other mills;[448-451] and in other effluent-exposed fish species sampled elsewhere.[442, 452, 453] The consequences of these similar endocrine alterations to whole animal reproductive fitness and population dynamics,

however, have varied greatly among species. For example, longnose sucker exposed to BKME show no organism responses other than an altered age distribution, whereas white sucker and lake whitefish show decreased gonadal sizes, secondary sexual characteristics, and egg sizes, and increased age to maturity.[442] In a review of whole organism responses of fish exposed to different kinds of mill effluents (including unbleached pulps), 80% showed increased age to sexual maturation, and reduced gonadal size was reported in 58% of the studies.[454] These observations provide evidence for species differences in susceptibility to BKME, but also show the inherent difficulty when trying to compare biological responses in fish populations inhabiting highly different environments and exposed to complex mixtures likely to vary in chemical composition.

There are relatively few studies on the effects of BKME on egg and fry parameters, and the results from these studies are conflicting. Fertilities (as indicated by the percentage of spawned eggs that hatched) were decreased in zebrafish (*Danio rerio*) after exposure to chlorinated phenolics from a bleach plant effluent[455] and in brown trout *(Salmo trutta*) after exposure to BKME.[456] Hatchabilities were also reduced in pike (*Esox lucius*) after exposure of eggs to BKME concentrations as low as 0.5%.[457] Similarly, many field and laboratory studies have reported declines in fecundities of several fish species after exposure to paper mill effluents.[445, 445, 448, 455, 458, 458-460] McMaster et al. [461] on the other hand, found equal or greater fertilization rates and no effects on hatchabilities of white suckers eggs, despite declines in sex steroid concentrations, gonad and egg sizes and sperm motility in BKME-exposed fish. In addition, fecundities and/or hatchabilities were not altered after exposures to BKME in several other field[452, 462, 462, 463, 463] and laboratory studies.[453, 464] There is very little information on the developmental effects of BKME. In the laboratory, survival from larvae to adult and growth of fathead minnows (*Pimephales promelas*) were not affected after exposures to up to 20% effluent concentrations.[458, 464, 465] Studies conducted in our laboratories with largemouth bass (*Micropterus salmoides*), however, have found similar fecundities and hatchabilities, but decreased fry survival after exposures to 10% BKME.[453] Similarly, Karås et al.[463] reported comparable fecundities and egg mortalities in perch (*Perca fluviatilis*) from a BKME-exposed area, but fry hatched from this site were smaller and had an increased frequency of abnormalities which was translated into lower abundances of fry and young-of-the-year fish. These authors concluded that exposure of perch to BKME had resulted in high mortality rates close to the time of hatching due to either chronic failure of parental reproductive systems and/or acute toxicity to embryos or early larvae.

Exposure to pulp and paper mill effluents has also been associated with alterations in secondary sex

characteristics (see[466]for a review of the evidence for masculinization in poeciliids from Florida). Female mosquitofish, *Gambusia affinis*, inhabiting a stream receiving paper mill effluents in Florida were reported to be strongly masculinized showing both physical secondary sex characteristics (fully developed gonopodium) and reproductive behavior of males.[467] More recently, masculinization of female fish has been identified from an additional two species (least killifish, *Heterandria formosa* and sailfin molly, *Poecilia latipinna*) collected from an effluent-dominated stream.[468] Masculinization of female fish has been attributed to the action of androgenic hormones that result from the biotransformation of plant sterols (and also cholesterol and stigmasterol) by bacteria such as *Mycobacterium*. [469]

Results from studies on white sucker indicate that several sites within the pituitary-gonadal-axis are affected after exposure to BKME. Fish from exposed sites had significantly lower plasma levels of gonadotropin (GtH-II) and showed depressed responsiveness of sex steroids and 17,20ß-dihydroxy-4-pregnen-3-one (a maturation-inducing steroid) after GnRH injections.[470] BKME-exposed fish also had lower circulating levels of testosterone glucoronide, which would be suggestive of altered peripheral steroid metabolism. Similarly to what was observed under in vivo conditions, in vitro incubations of ovarian follicles collected from BKME-exposed females have also shown reduced steroid production.[443, 470] The similarities between both types of studies would suggest that reductions in plasma steroid levels in BKME-exposed fish are mainly due to alterations in ovarian steroid production. Recent studies on white sucker have shown increased apoptotic DNA fragmentation and increased expression of a 70-kDa heat shock protein in oocytes from prespawning females, which coupled with lower sex steroids may explain the observed decreased gonad weights and delayed sexual maturity. [471]

Although there is extensive literature on the reproductive effects of BKME on fish, very little is known about the chemical compound(s) that could be held responsible for such changes. Compounds such as dioxins and furans were the first to blame, because of their persistence, bioaccumulative properties, and their known deleterious reproductive and antiestrogenic effects.[472] Recent evidence, however, suggests that the chemical(s) in pulp mill effluents responsible for reproductive alterations are relatively short-lived and readily metabolized by fish. For example, mixed-function oxygenase induction and endocrine alterations have also been reported downstream from mills that do not use chlorine bleaching,[442] and these parameters have rapidly returned to normal after cessation of exposure.[444] Indeed, several of the natural wood components in the final effluent, such as sterols, lignans, stilbenes, and resin acids, are believed to be weak estrogens.[473, 474] For example, the plant sterol, β-sitosterol, has demonstrated

estrogenic activity by its ability to induce VTG in juvenile rainbow trout[474] and male goldfish[475] and bind to the ER in rainbow trout hepatocytes[476] Conversely, various phytosterols that survive the treatment process have displayed masculinizing effects under controlled experimental conditions.[477]

Sewage-treatment effluents

English researchers have shown that effluents coming from sewage-treatment plants might cause estrogenic effects in fish, due to their ability to induce the production of VTG (a female specific egg-yolk precursor) in males (see [478] and [479] for a review on this topic). Recent information has also shown an increase in the incidence of intersex or hermaphroditism in populations of wild fish inhabiting rivers contaminated with sewage effluent.[480] Presently, the population-level effects of increased VTG in male fish remain poorly understood, although they are known to be associated with decreased testicular growth.[481] [482] Chemical analysis of effluents from sewage-treatment plants has identified several compounds with estrogenic properties, including: natural estrogens (E_2 and estrone); synthetic estrogens (17α-ethynylestradiol or EE2, widely used in birth control pills); alkylphenolic chemicals (resulting from the breakdown of non-ionic surfactants); plasticisers (bisphenol-A); and phathalates.[481, 483, 484] The following section reviews the major findings on the endocrine-disrupting effects of the above groups of chemicals in fish.

Natural and Synthetic estrogens. Recent studies using chemical fractionation and biologic screening techniques, suggest that natural and synthetic steroidal estrogens may be causing the greatest estrogenic effects in fish inhabiting streams contaminated with sewage-effluents.[485] This stems from the fact that both types of estrogens, but especially the synthetic ones, are highly potent hormones, and thus concentrations in the pptr or less are capable of inducing biological effects. For example, EE_2 induces VTG synthesis in male rainbow trout at concentrations as low as 0.1 pptr.[486] EE_2 concentration in English rivers has ranged from 0.2 – 7 pptr.[487] Although the reproductive consequences of EE_2 exposure in fish are mainly unknown at this time, they have been associated with decreased testicular growth and development in immature fish.[488] Altered spermatogenesis has also been reported in fish exposed to natural estrogens.[489] Women are the primary sources of natural and synthetic estrogens in sewage effluents, which get excreted as inactive conjugates during menstrual cycling, or because of the use of contraceptive pills. During the sewage-treatment process, these conjugates are biotransformed into their parent and biological active compounds.

Alkyl phenol ethoxylates (APEs) and Alkyl phenols (APs):

APEs are effective non-ionic surfactants, serving as components of industrial and domestic detergents, pesticide formulations, cosmetics, and paints. Of all APEs produced, nonylphenol-polyethoxylates and octylphenol-polyethoxylates constitute approximately 80% and 20%, respectively. These chemicals are biodegraded during sewage treatment to form APs, such as nonylphenol and octylphenol. Industrial effluents might contain over 100 ppb of nonylphenol, although most streams surveyed in the UK and in the US contained equal or less than 10 and 0.1 ppb, respectively.[490, 491] Nonylphenol and octylphenol, are hydrophobic and lipophilic and thus can accumulate in sediment and fish adipose tissue. Both APEs and APs are known to have estrogenic properties, as discussed below.

Alkylphenolic chemicals might also be playing an important role as xenoestrogens in sewage effluents. Male rainbow trout exposed to four AP chemicals, responded with significant increases in plasma VTG concentrations, particularly after treatment with at least 3 ppb octylphenol.[481]. Nonylphenol and two carboxylic acid APE degradation products also induced VTG production in males in this study, but at higher concentrations. Testicular growth was inhibited in response to all four chemicals, with octylphenol having the greatest inhibitory effect. Christianson et al.[492] reported similar effects in male eelpout (*Zoarces viviparous*) exposed to nonylphenol. Twenty-five days after a 10-100 ppm nonylphenol injection, a significant increase in plasma VTG with a concomitant decrease in GSI was observed. Histological examination revealed degenerated seminiferous lobules in exposed males, as well as decreased GTP activity (a marker for Sertoli cell function). Plasma VTG induction has also been reported following nonylphenol exposure in male and immature female rainbow trout,[493] male flounder,[494] male and female Atlantic salmon, [495, 495, 496] Japanese medaka,[495, 497] and immature channel catfish. [498] Nonylphenol (25 ppm) has also caused a dramatic increase in plasma zona radiata proteins in juvenile female Atlantic salmon.[496] Gray et al.[499] recently reported a reduction in courtship activity in adult Japanese medaka males exposed to octylphenol from 1 day posthatch to 6 months posthatch. In this study, transgenerational effects were also observed (i.e. an increase in fry developmental abnormalities). Similarly, AP-induced developmental toxicity effects have been reported in embryos and larvae of killifish (*Fundulus heteroclitus*) after exposures to octylphenol and 4-tert-octylphenol.[500] Disruption of sexual differentiation is yet another effect observed in fish exposed to APs, having been reported in common carp[501] and mosquitofish.[502]

The endocrine-disrupting properties of APEs and APs are mainly related to their ability to bind to the ER. Indeed, APEs have been shown to be estrogenic using several in vitro bioassays.[503 504, 505] Similarly, APs substituted at position 4 (e.g., 4-nonylphenol) have demonstrated estrogenic activity in various in vitro and in vivo bioassays. [506-508 503 509] P-substituted phenols, such as 4-t-pentylphenol (TPP), are believed to be among the most potent estrogens.[508, 510] Ren et al.[511] suggested that nonylphenol may also be involved in the post-transcriptional regulation of VTG mRNA processing. Finally, recent research shows that APs can induce reproductive alterations through an increase in the rate of apoptosis of Sertoli cells, phenomena that can negatively affect the development and release of sperm.[512]

Bisphenol A: Bisphenol is the generic name given to a group of diphenylalkanes commonly used in the production of plastics. Bisphenols consist of two phenolic rings joined by a carbon bridge. The bridging carbon has no substituent in bisphenol F and two methyl groups in bisphenol A (BPA). Incomplete polymerization or depolymerization of plastics from heating may result in the release of BPA into the environment and subsequent human and animal exposure. The first reports to document the estrogenic potential of the bisphenols appeared in the 1930s,[513] and a number of investigators employing a variety of techniques have since confirmed those results (e.g., [514 515]). Evidence suggests that estrogenic potency of these compounds increases with the length of the alkyl substituent at the bridging carbon, as well as the chemical nature of the substituents.[516] Bisphenols with hydroxyl groups in the para position and an angular conformation are suitable for binding the ER at the acceptor site.

The estrogenicity of BPA has been demonstrated by several in vitro assays (e.g., [409, 506, 510, 517-520]). More recently, BPA has been shown to induce the synthesis of the VTG protein in rainbow trout liver slices;[521] VTG mRNA in rainbow trout primary hepatocyte cultures;[522] and VTG and zona radiata proteins in Atlantic salmon primary hepatocytes.[523] In the latter study, BPA inhibited the E_2-stimulated induction of VTG and zona radiata proteins, suggesting that the effects of the plasticizer are truly estrogenic in nature. Arukwe et al.[496] observed a dose-dependent increase in plasma VTG and zona radiata proteins following a single intraperitoneal injection of bisphenol A.

Interestingly, recent evidence suggests that the in vivo estrogenicity of BPA may be greater than predicted by in vitro assays.[524] Male Japanese medaka were exposed to BPA for two weeks and then introduced to a tank with untreated females for spawning studies.[497] In the experimental group, the number of hatchings was reduced, and the

concentrations that affected reproduction in this study were lower than concentrations that produced effects in some in vitro studies. Furthermore, VTG synthesis was observed at concentrations below those affecting reproduction. Bisphenol A is also known to cause significant declines in sperm production.[425, 525]

Other phenolics: Several phenolic compounds, other than alkyl phenols and BPA, have been evaluated for their impact on fish reproduction. For example, polychlorinated phenols are often formed during the chemical reaction of chlorine and phenolic compounds in wood pulp. The polychlorinated phenols are acidic and are chemically reactive compounds of low persistence because of their water solubility. Pentachlorophenol (PCP), a commonly used fungicide in wood preservation, often enters the environment as a component of domestic and industrial effluents, primarily from the forest products industry.

There is evidence that reproductive effects can be elicited when fish are exposed to PCP. Female rainbow trout exposed for 18 days to sublethal concentrations of PCP (22 and 49 ppm) during the primary ovarian growth phase displayed a significant increase in oocyte atresia and a trend toward decreasing oocyte diameter.[526] The use of purified PCP in this study rebutted the claim that toxicity of technical PCP is due to contamination by PCDDs, PCDFs, or other chlorinated phenols.[527] It has been suggested that PCP affects oogenesis by interfering with the production of yolk in the liver of rainbow trout.[528] These authors found that PCP may act as an estrogen antagonist, since it has shown a slight inhibitory effect on E_2-stimulated induction of the ER mRNA and a substantial inhibitory effect on E_2-stimulated induction of VTG mRNA. In addition, a study with *Daphnia magna* found that PCP is capable of altering steroid hormone biotransformation and elimination pathways.[529] The potential estrogenicity of several other phenolic compounds was tested by[483] using a trout ER competition study and several mammalian cell assays. 2,4-dichlorophenol, a component of fungicides and germicides, reduced the binding of radiolabeled E_2 to the trout ER. Conversely, 3,4-dimethylphenol and 2-methylphenol, which also serve as fungicides and disinfectants but have no chlorine group, failed to compete for ER binding. In another study,[530] examined the effect of phenol on the steroidogenesis and reproductive activity in sexually maturing carp. After 48 days of exposure to 8 ppt phenol, GSI was reduced, ovarian and liver cholesterol concentrations were increased, and cholesterol conversion to sterol products was inhibited. Previous studies by Kumar and Mukherjee[531] also demonstrated phenol-induced alterations in plasma, ovarian, and hepatic cholesterol concentrations in several species of fish.

3.7 *Metals:*

Although metals are natural substances, human activity is largely responsible for their abnormal release and accumulation in the environment. Metal toxicity usually results from exposure to high levels of non-essential metals such as mercury (Hg) or cadmiun (Cd). Since these and all other metals are non-biodegradable; the body cannot metabolize them into less toxic forms. Instead, detoxification involves binding to specific proteins (e.g. metallothionein) that function to shield toxic properties or to produce insoluble forms (e.g. intracellular granules) for long-term storage or excretion. If not excreted, metals can bioaccumulate in tissues, especially if the individual occupies a position at the top of the food chain. Metals probably do not act as classic EDCs i.e.modulating receptor-mediated effects. Instead, their mechanism of action may involve toxicity of endocrine tissues, altered enzyme binding, or central nervous system interactions. Nonetheless, exposures may result in changes in concentrations of serum hormones producing some deleterious change in either the adult or developing embryo.

Mercury:

Mercury is a non-essential heavy metal found naturally in the environment and used in many industries, including battery, paper, paint, chemical, and agriculture, as well as dentistry and medicine. The burning of coal, natural gas, and refining of petroleum products adds 5,000 tons of mercury per year to the atmosphere increasing mercury contamination of aquatic ecosystems worldwide.[532, 533] Mercury enters aquatic systems either indirectly by atmospheric deposition or from direct discharge of mercuriferous wastes into watersheds.[534, 535] Conditions of low pH and high dissolved organic carbon increase the methylation of inorganic Hg to the more toxic methyl mercury (MeHg). [536] This methylated form is rapidly bioaccumulated by aquatic species with body burdens in piscivores increasing with trophic level.[537] While there have been many studies measuring Hg concentrations in wildlife[538-543] [544] little information is available on its potential effects.

In laboratory tests Hg has produced stillbirths in dogs and pigs and abortions, [545] #779} abnormal sperm ,[546] and low conception rates in monkeys. [547] Mercury ladened rats have reduced litter size[548] and decreased surviva. [549] In mice, Hg produces decreased fetal survival,[550] fetal malformations,[551] embryo resorption.[552] low sperm count,s [553] and tubular atrophy of testes.[554] Abortions have been reported in guinea pigs[555] [556] and Hg in the Florida panther is thought to reduce kitten survival.[557] These effects in offspring are expected

because Hg (both inorganic and organic) is able to cross the placenta producing behavioral deficits, impaired fertility and fetal death.[558, 559]

Studies in birds have been limited to evaluating effects on hatchability and survival. Some studies have found a negative correlation between hatching success and Hg concentrations in eggs [543, 560, 561] or feathers.[562, 563] Common tern with liver Hg concentrations between 9 - 21 ppm wet wt showed decreased hatchability and reduced nesting success.[564] Concentrations between 3 -14 ppm in common loons decreased hatchability and at 52 ppm reduced nesting success.[560] In this same study, brain concentrations > 2 ppm reduced egg laying and decreased nest and territory fidelity.[560] Egg concentrations from 0.5 to 1.5 ppm wet wt decreased hatchability in pheasants.[565, 566] Mallard eggs with externally applied MeHgCl showed decreased embryo weights, developmental abnormalities, and embryonic death. Juvenile survival was also decreased in these studies because of neurological damage. Ducks fed Hg over three generations had decreased reproduction and altered behavior in the ducklings. [567]

There is little information on Hg reproductive toxicity in reptiles and amphibians. The amphibian *Pleurodeles waltl* raised in water with low concentrations of MeHgCl showed chromosome breaks.[568] Frogs (*Rana cyanophlyctis*) kept in water for less than three months had decreased GSI as well as reduced numbers of sperm bundles and increased secondary spermatogonia indicating a blockage in mitosis and thus in the conversion of spermatogonaia into primary spermatocytes.[569] Mercury contamination is also reported to have caused a loss in germ cells and sterility in *Rana nigromaculata* [570] and reduced survival in *Xenopis laevis* .[571]

In fish, Hg exposure has caused decreased GSI and a variety of gonadal abnormalities.[572] Other responses to exposure involve altered lipid and cholesterol ovarian content;[573] reduced spermatogenesis;[346] and impaired fertilization.[574, 575] Studies with tilapia have shown reduced plasma E_2 in females and plasma 11KT in males with muscle concentrations ranging from 1 to 7 ppm wet wt (Arnold et al. in preparation). Similar effects have been seen in largemouth bass with muscle Hg as low as 0.25 ppm wet wt.[109] These studies suggest a central nervous sytem expsoure and subsequnet effects on the hpothalamic-pituitay-gonadal axis in fish.

Mercury may depress hormone production by acting on the gonads and interfering with their development. Testicular atrophy was observed in tilapia (*Oreochromis niloticus*) with Hg concentrations between 0.4 – 2.7 ppm dry wt. and in guppies (*Poecdillia reticulata*).[576, 577] Another possible mechanism for hormone disruption is interference with the brain-hypothalmus-pituitary-gonadal axis. If mercury interferes with the production of

Gonadotropin Releasing Hormone (GnRH) or Gonadotropin Hormone (GTH) there would be no hormonal stimulus for further gonadal development.

Other Metals

Lead (Pb) is a heavy metal released into the atmosphere from industrial emissions and motor exhaust. It is a nonessential, toxic metal that affects all body systems. Once ingested it is deposited in mamamls in the following order: bone, kidney, liver, brain, and muscle. Reproductive effects in mammals inclued alterations in implantation, embryonic development, and on reproductive organs.[578, 579 580] found that blood levels of Pb > 39 ug/dl in male rats produced prostatic hyperplasia, impared sperm motility, reduced testicular weight, seminiferous tubular damage, and spermatogenic cell arrest. Lead is also capable of crossing the blood-brain barrier, interfering with the central nervous system.[581, 582] Young can also be exposed through the maternal milk.[583] The fetus and developing young are most sensitive to chronic levels of Pb exposure. Lead blood levels as low as7 to 8 ug/dl can result in neurobehavioral symptoms.[584, 585] In prenatal stage Pb that passed through the placenta is linked to reduced gestational age and lowered birth weight.[586]

Birds become contaminated with Pb through the consumption of lead shot or bullets or fishing sinkers. These items gradually dissolve and the birds become progressively weaker and emaciated. Other species, especially raptors which may prey off of these species get lead poisoning by ingesting the contaminated prey.[587] There is little research on the reproductive effects of Pb in reptiles. Turtles (*Trachemys scripta*) injected with Pb had reduced righting response[588] and this heavy metal reduced the rate of development of the Jefferson salamander (*Ambystoma jeffersonianum).*[589] Although known to accumulate in fish tissues,[590] few studies have examined the effects of Pb exposure on fish reproduction or endocrine functions. Female Atlantic croaker fed as little as 0.05 – 0.2 ppm/day for one month had reduced GSI, E_2, and T.[141, 142] Female climbing perch, *Anabas testudineus*, exposed via water to 1.25 ppm Pb for one month had lower GSI, [591] and retarded ovarian growth was observed in Clarias catfish following long-term exposure (275 days) to 5 ppm.[592] In addition, decreased spermatogenesis and ovarian atresia have been observed ir Rosy barb exposed to a low dose of Pb nitrate (0.12 ppm) for 60 – 120 days,[593] and decreased spermatogenesis and hemorrhage in the testis was reported in *Colisa fasciatus* following four days of exposure to 15 ppm Pb nitrate [594].

Copper (Cu) is an essential metal that is necessary for the activity of various enzymes and

for iron utilization. This metal has received widespread use in the preservation and coloring of foods, in brass and copper water pipes and domestic utensils, and in fungicides and insectides; the latter providing the primary route of exposure to aquatic animals. Egg and larval mortality of the Jefferson salamander were decreased by exposure to Cu.[589] Several studies involving different fish species report a spectrum of reproductive abnormalities following exposure to Cu. Decreased spermatogenesis and ovarian atresia were observed in female rosy barb, *Puntius conchonius,*[593] whereas testicular abnormalities and arrested spermatogenesis were observed in male *Lebistes reticularis.*[595] Although VTG is known to serve as a carrier for many metals, including Cu, the effects of this metal on vitellogenisis are not clear. Copper suppressed vitellogenesis in female *Mytilis edulis,*[596] whereas cupric acetate appeared to have no effect on vitellogenesis in female Clarias catfish.[592] Additional studies involving Cu exposure report decreased egg size and a propensity for deformities on larvae of white sucker, (*Catostomus commersoni);*[597] reduced egg viability and hatchability in brook trout; [598] and impaired fertilization and increased larval abnormalities in *Atherinops affinis.*[599]

Cadmium (Cd) is a byproduct of Pb and Zn mining and is found in industrial sludges and phosphate fertilizers. Cd has been reported to accumulate primarily in the liver and kidneys,[600 601] however, several investigators have also detected Cd in the gonads.[602, 603] There is no data on reproductive effects in mammals however, Cd suppresses egg production.in mallards[604] and chickens[605] Seabirds with concentrations of 40 - 100 ppm in liver showed no signs of impaired egg production suggesting that chronically exposed seabirds have developed resistance to the effects of this metal.[606] There are no studies of egg shell thinning in wild bird populations.[606] Slight gonadal alterations were found in mallards fed Cd and accumulating kidney concentrations up to 50 ppm while those with kidney concentrations of 100 ppm had testicular atrophy and no sperm production.[607] Again, actively reproducing seabirds have been found with similar kidney concentrations. [606] In amphibians, studies with *Xenopus laevis* showed that females exposed to Cd for 4 w produced malformed embryos. [608] Malformations were also seen in *Xenopus* embryos exposed to concentrations from 0.1 to 10 mg Ca^{2+}/L. This study showed that embryos were more susceptibile from stages 2 to 40, although malformations occurred at all stages.[609]

Diverse effects of Cd exposure have been reported in a number of fish species, although results from different studies are occasionally conflicting. For instance, Cd exposure led to reduced plasma sex steroids, in *Monopterus albus* and brook trout (*Salvelinus fontinalis)*; reduced VTG in *Monopterus albus*, rainbow trout,

bleeker (*Lepidocephalicthys thermalis)*, winter flounder (*Pleuronectes americanus),* and flounder (*Platichthys flesus)*; and decreased GSI in *Monopterus albus* and winter flounder.[144, 610-614] Conversely, Cd appeared to stimulate steroidogenesis in brook trout,[615] Atlantic croaker,[142, 616] and rainbow trout.[617] In addition, Cd increased the in vitro production of GTH, which was consistent with the enhanced ovarian activity observed in female Atlantic croaker.[616] A wide range of Cd concentrations (0.001-1000 ppm) and durations of exposure (several hours to 90 d) were used in the experiments described above, which could explain the different responses observed.

Cadmium treatment has been associated with degenerative changes in the gonads of several fiah species.[595, 612, 618, 619] Adult female guppies exposed to dietary Cd for 30-120 d produced less fry compared with controls, demonstrating the effect of Cd at the organism and possibly at the population level.[620] The mechanisms underlying Cd-induced alterations are poorly understood, although several theories have emerged. There is speculation that vitellogenesis may be impaired because the synthesis of metallothioneins by the liver in response to metal exposure takes priority over the synthesis of VTG.[614] On the other hand, Cd may directly interfere with VTG synthesis at the transcriptional or translational levels. Others suggest that Cd may interfere with the incorporation of VTG into the developing oocyte. In a study by Victor *et al.*,[612] Cd appeared to impede the transport of VTG across the oolemma into the oocyte. However, Cd-VTG complexes injected into Atlantic croaker were shown to incorporate into the ovaries.[602]

Zinc is a component of over 70 metalloenzymes and serves as an important essential metal. Although Zn toxicity is rare, it has been reported in several species. For instance, Zn has been shown to influence hatching success and developmental rates in the Jefferson salamander.[589] Multiple studies also document reproductive alterations in fish following Zn exposure. Purified VTG from E_2-treated male red drum was found to contain Zn,[602] and in a separate study using clarias catfish, Zn was reported to decrease circulating levels of VTG.[592] Other observed effects in fish include delayed spawning and decreased egg viability in zebrafish;[621] impaired spermatogenesis and increased oocyte atresia in rosy barb;[622] and reduced egg size and increased larval deformities in white sucker.[623]

Selenium (Se) is a natural element/metal required for healthy nutrition in small amounts, but toxic at higher concentrations. The processing of fossil fuels releases Se to the environment which accumulates in coal fly ash. Selenium is also found in high concentrations in certain soils, remaining in wetlands as a byproduct of

irrigation.[624, 625] Reproductive success of birds and fish is more sensitive to selenium toxicity than are growth and survival of young or adults.[626, 627] In fish, Se exposure can result in subtle but dramatic reproductive failure.[626] In birds, Se egg concentrations of 3 ppm wet wt are considered the threshold for reproductive impairment. [628-630]

4.0 SUMMARY and CONCLUSIONS:

This chapter has reviewed and selectively summarized the current evidence for potential endocrine disrupting effects of specific chemicals and chemical classes in vertebrate wildlife and their potential modes of action. Although evidence of endocrine disruption in wild species has accumulated during recent years, most studies are based on indirect evidence rather than defined mechanisms and exposures to specific ECDs. Indeed, most studies of potential EDC effects in wildlife are based upon observed adverse reproductive and developmental effects rather than direct evidence of endocrine-modified function and/or defined endocrine pathways. Nonetheless, a consideration of whether the effects of specific chemicals can be attributed to hormonal properties, mechanisms or pathways is critical to the identification of a chemical as an EDC or EAA.

This review also evaluated the evidence for endocrine disruption for wildlife and fish in field/natural and control/experimental evaluations. A wide variety of chemicals have been reported as potential EDCs in wildlife. The major chemical classes summarized here include, but are not limited to: (PAHs, PCBs and PBBs, PCDDs and PCDFs, organochlorine pesticides, non-organochlorine pesticides, complex environmental mixtures, and selected metals. In addition, the evidence of potential EDC effects are summarized and reviewed for multiple vertebrate species, with an emphasis on reproductive and developmental effects, which are often modulated by endocrine mechanisms and pathways. Collectively, there is strong evidence of altered reproductive and developmental processes in wildlife exposed to potential EDCs. Although from most of these studies the mechanisms of action and direct link to endocrine mediated pathways are often times unclear, there is generally evidence for an association between effects and chemical/contaminant exposures, as well as evidence of effects in multiple vertebrate classes. Much of the evidence for EDC effects in wildlife is derived from observations and studies involving fish. These studies present the clearest link between environmental chemical contaminants and endocrine disrupting effects. The potential mechanisms of action are diverse (see Figure 1) and endocrine/hormonal mediated pathways are likely.

In recent years great progress has been made in the development of in vitro screening and testing procedures for the identification of potential EDCs. However, these assays have been based primarily on receptor mediated responses and hormone mimicry. It is important to mention however, that a wide variety of other potential mechanisms also exist for EDCs (see Figure 1 and Table 1) and thus there is a strong need for the development of additional screening and testing procedures. On the other hand, in vivo studies are not only more ecorelevant, but also more useful for the assessment of risk in wildlife due to EDCs. However, the interpretation of effects at the organism level and above is difficult and potentially affected by multiple stressors (other than EDCs). Paired studies, involving both field and laboratory-based exposures, as well as in vitro assessments of mechanisms are likely needed to adequately identify and evaluate potential EDCs. Nonetheless, studies in wildlife and fish have provided the strongest evidence for accepting the endocrine disrupting hypothesis and has been critical in the identification and evaluation of potential environmental EDCs.

Table 1. Summary of effects and possible modes of action (MOA) of endocrine disrupting chemicals (EDCs), by chemical class and taxa.

Chemical (s)	Taxa	Effects	Possible MOA	Sample Reference
PAHs				
	Birds	↓hatchability	DNA damage; oxidative stress; ER agonist	134
	Fish	↓GSI impaired gonadal deveolpment	DNA damage; oxidative stress; ER agonist	149 146
PCBs				
	Mammals	abortions & stillbirths	Antiestrogens; Act through Ah receptor	167
	Birds	↓eggshell thickness ↓hatching success ↑embryo mortality	Antiestrogens, Act through Ah receptor	182 188
	Amphibians and Reptiles	↓testosterone ↑mortality & malformation rates	unknown	203 631
	Fish	↓spawning ↓hatchability	Antiestrogens, Act through Ah receptor	206 632
PBBs				
	Mammals	fetotoxic and teratogenic - rats ↑menstrual cycles, ↓progesterone levels	unknown	226 633 634
	Birds	↓offspring viability ↓hatchability	unknown	227

Table 1. Countinued

Organochlorine pesticides & fungicides				
Cyclodienes	Birds	↓productivity	E_2	289, 290, 635, 636
	Reptiles	sex reversal ↓plasma hormones	E_2 agonist	637 203
	Fish	↓fertililzation ↓maturing oocytes altered spermatogenesis	E_2 agonist	638 639 301
Chlordecone and mirex	Mammals	reproductive impairment	weakly estrogenic	311
	Birds	↓clutch size, egg size, shell thickness, hatchability ↑embryo malformations		310
	Fish	gonadal abnormalities		325
DDT and derivatives	Mammals	persistant vaginal estrus	Androgen antagonist	327
	Birds	eggshell thinning reproduction problems population reduction		640 641
	Reptiles and Amphibians	sex reversal ↓clutch viability, altered plasma hormone levels, abnormal gonadal morphology	hormone mimicy estrogenicity steroid receptors	642 201, 643
	Fish	↑oocyte atresia, ↓fecundity and fertility ↓plasma hormones	hormone mimicy estrogenicity steroid receptors	340 344

Table 1. Continued

Hexachloro-cyclohexane lindane	Mammals	interfers with estrogen function	antiestrogenic, weakly estrogenic	644
	Birds	oocyte atrophy, ↓vitellogenin		645 646
	Fish	↓plasma hormones		647
Vinclozolin				
	Mammals	feminization of males	androgen antagonist	648
PCDDs & PCDFs TCDD				
	Mammals	impairs sexual differentiation in male rats, delay in testicular descendent and puberty	Antiestrogenic through AR Receptor	235 236
	Birds	developmental alterations congenital deformities feminization	antiestrogenic	239 240
	Amphibians and Reptiles	early metamorphosis ↑Frequency of deformities alterations in sex ratios	antiestrogenic	256 258 259
	Fish	early life stage mortality impaired oocyte development	antiestrogenic	649

Table 1. Continued

Non-organochlorinated pesticides				
Organophosphate pesticides (OP)	Mammals	depressed reproduction	acts at sites on hypothalamus-pituitary-gonadal-liver axis; acetylcholinesterase inhibitor	358
	Birds	altered gonadotrophins developmental defects		361 134
	Amphibians and Reptiles	altered metamorphosis ↑deformities and delayed development		372 373
	Fish	retarded ovarian growth ↓GSI arrested spermatogenesis		376
Carbamate pesticides	Amphibians	↑developmental deformities	acetylcholinesterase inhibitors acts on pituitary to alter GnRH and GTH concentrations	402
	Fish	↑histopathological alterations in gonads ↓GSI ↑oocyte atresia ↑spermatogonial necrosis		381 382
Organometal pesticides-(TBT)	Fish	↓sperm counts delayed hatching	may inhibit aromatose	425 419
	Invertebrates	masculinization of gastropods	competitive inhibitor of aromatase, cytotoxic and genotoxic effects	411

Table 1: Continued

Complex Mixtures				
Pulp and Paper Mill Effluents	Fish	↓steroid bisynthesis, ↓gonadal development, delayed sexual maturation, altered secondary sex characteristic expression	estrogenic ER, AR, AhR agonists	56 650
Sewage-treatment effluents	Fish	↓testicular growth & development altered spermatogenesis	estrogenic ER agonists/antagonist	481 489
		↑VTG production in males ↓hatchlings ↑oocyte atresia	estrogenic ER binding	481 425, 492, 497, 526
Methylmercury				
	Mammals	↓embryo survival ↓sperm counts	unknown	549 553
	Birds	impaired reproductive behavior ↓hatchability & nesting success	unknown	564, 651
	Amphibians and Reptiles	↓GSI ↓sperm bundles		652
	Fish	↓GSI ↑gonadal abnormalities Altered gonadal steroidogenesis	unknown	653

References

1. **Kavlock, R.J. and Ankley, G.T.**, A Perspective on the Risk Assessment Process for Endcorine-Disruptive Effects on Wildlife and Human Health., *Risk Analysis,* 16, 731, 1996.

2. **Kavlock, R.J., Daston, G.P., DeRosa, C., Fenner-Crisp, P., Gray, L.E., Kaattari, S., Lucier, G., Luster, M., Mac, M.J., Maczka, C., Miller, R., Moore, J., Rolland, R., Scott, G., Sheehan, D.M., Sinks, T., and Tilson, H.A.**, Research Needs for the Risk Assessment of Health and Environmental Effects of Endocrine Disruptors: a Report of the US EPA Sponsored Workshop., *Environ. Health Persp.* 104, Supplement 4, 715, 1996.

3. **Colborn, T., Von Saal, F.S., and Soto, A.M.**, Developmental Effects of Endocrine-Disrupting Chemicals in Wildlife and Humans., *Environ. Health Persp.* 101, 378, 1993.

4. **Tyler, C.R., Jobling , S., and Sumpter, J.P.**, Endocrine Disruption in Wildlife: A Critical Review of the Evidence, *Crit. Rev. Tox.*, 28, 319, 1998.

5. **Dawson, A.**, Mechanisms of Endcorine Disruption With Particular Reference to Occurrence in Avian Wildlife: A Review., *Ecotoxicology*, 9, 59, 2000.

6. **Iguchi, T. and Sato, T.**, Endocrine Disruption and Developmental Abnormalities of Female Reproduction., *Am. Zool.*, 40, 402, 2000.

7. **Harrison, P.T.C., Holmes, P., and Humfrey, C.D.N.**, Reproductive Health in Humans and Wildlife: Are Adverse Trends Associated With Environmental Chemical Exposure?, *Sci. Total Environ.*, 205, 97, 1997.

8. **Depledge, M.H. and Billinghurst, Z.**, Ecological Significance of Endocrine Disruption in Marine Invertebrates., *Mar. Pollut. Bull.*, 39, 32, 1999.

9. **Sumpter, J.P.**, Xenoendocrine Disrupters - Environmental Impacts., *Toxicol. Lett.*, 103, 337, 1998.

10. **Datson, G.P., Gooch, J.W., Breslin, W.J., Shuey, D.L., Nikiforov, A.I., Fico, T.A., and Gorsuch, J.W.**, Environmental Estrogens and Reproductive Health: a Discussion of the Human and Environmental Data., *Reprod. Toxicol.*, 11, 465, 1997.

11. **Kendall, R.J., Dickerson, R.L., Geisey, J.P., and Suk, W.P.**, *Principles and Processes for Evaluating Endocrine Disruption in Wildlife.* SETAC Press, Pensacola, FL, 1998, <25 Page(s)>.

12. **USEPA**. *EPA Special Report on Endocrine Disruption.*, Fact Sheet. USEPA, Washington, D.C., 1997.

13. **National Research Council**, *Hormonally Active Agents in the Environment.* National Academic Press, Washington, D.C, 1999, <25 Page(s)>.

14. **USEPA.** *Endocrine Disruptor Screening and Testing Committee (EDSTAC) Final Report.*, USEPA, Washington, D.C., 1998.

15. **Licht, P.**, Reproductive Endocrinology of Reptiles and Amphibians: Gonadotropins. *Annual Review of Physiology,* 41, 337, 1979.

16. **Norris, D.O.**, *Vertebrate Endocrinology.* Academic Press, San Diego, CA, USA., 1997, <25 Page(s)>.

17. **Norris, D.O. and Jones, R.E.**, *Hormones and Reproduction in Fishes, Amphibians and Reptiles.* Plenum Press, New York, NY, USA., 1987, <25 Page(s)>.

18. **Peter, R.E., Yu, K.-L., Marchant, T.A., and Rosenblum, P.M.**, Direct Neural Regulation of the Teleost Adenohypophysis. *J. Exp. Zool.* 4, 84, 1990.

19. **Ben-Jonathan, N., Arbogast, L.A., and Hyde, J.F.**, Neuroendocrine Regulation of Prolactin Release., *Prog. Neurobiol.*, 33, 399, 1989.

20. **Peter, R.E.,** Vertebrate Neurohormonal Systems., in., Eds., Academic Press, New York, New York, USA, 1986, 57.

21. **Hirano, T.,** The Spectrum of Prolactin Actions in Teleosts, in *Comparative Endocrinology, Developments and Directives,* Ralph, C.L., Eds., Alan R. Lilss, New York, 1986, 53.

22. **Corpas, E., Harman, M., and Blackman, M.R.**, Human Growth Hormone and Aging., *Endocr. Rev.*, 14, 20, 1993.

23. **Shimoni, Y., Fiset, C., Clark, R.B., Dixon, J.E., McKinno, D., and Giles, W.R.**, Thyroid Hormone Regulates Postnatal Expression of Transient K+ Channel Isoforms in Rat Ventricle., *J. Phys.*, 500, 65, 1997.

24. **Muscat, G.E.O., Downes, M., and Dowhan, D.H.**, Regulation of Vertebrate Muscle Differentiation by Thyroid Hormone: the Role of the *MyoD* Gene Family., *BioEssays*, 17, 211, 1995.

25. **Pirinen, S.**, Endocrine Regulation of Craniofacial Growth., *Acta Odontol. Scand.*, 53, 179, 1995.

26. **Leonard, J.L. and Farwell, A.P.**, Thyroid Hormone-Regulated Actin Polymerization in Brain., *Thyroid,* 7, 147, 1997.

27. **Dodd, M.H. and Dodd, J.M.,** The Biology of Metamorphosis., in., Eds., Academic Press, New York, NY, USA, 1976.

28. **Hayes, T.B.**, Steroids As Potential Modifiers of Thyroid Hormone Action in Anuran Metamorphosis, *Am. Zool.*, 37, 185, 1997.

29. **Tata, J.R.**, Amphibian Metamorphosis: an Exquisite Model for Hormonal Regulation of Postembryonic Development in Vertebrates. *Dev. Growth. Differ.* 38, 223, 1996.

30. **Inui, Y., Yamano, K., and Miwa, S.**, The Role of Thyroid Hormone in Tissue Development in Metamorphosing Flounder., *Aquaculture*, 135, 87098, 1995.

31. **Tagawa, M., De Jesus, E.G., and Hirano, T.**, The Thyroid Hormone Monodeiodinase System During Flounder Metamorphosis., *Aquaculture,* 135, 128, 1994.

32. **Brown, D.D., Wang, Z., Furlow, J.D., Kanamori, A., Schwartzman, R.A., Remo, B.F., and Pinder, A.**, The Thyroid Hormone-Induced Tail Resorption Program During *Xenopus Laevis* Metamorphosis., *P. N.A.S. (US),* 93, 1924, 1996.

33. **Shi, Y.-B.**, Biphasic Intestinal Development in Amphibians: Embryogenesis and Remodeling During Metamorphosis., *Curr. Top. Dev. Biol.*, 32, 205, 1996.

34. **Hanken, J. and Hall, B.K.**, Skull Development During Anuran Metamorphosis II. Role of Thyroid Hormone in Osteogenesis. *Anal. Embryolology*, 178, 219, 1988.

35. **Hanken, J. and Summers, C.H.**, Skull Development During Anuran Metamorphosis: III. Role of Thyroid Hormone in Chondrogenesis. *J. Exp. Zool.* 246, 156, 1988.

36. **Buckbinder, L. and Brown, D.D.**, Thyroid Hormone-Induced Gene Expression Changes in the Developing Frog Limb. *J. Biol. Chem.* 267, 25786, 1992.

37. **Norris, D.O. and Platt, J.T.**, T3 and T4-Induced Rates of Metamorphosis in Immature and Sexually Mature Larvae of *Ambystoma Tigrinum*, *J. Exp. Zool.* 189, 303, 1974.

38. **Dickhoff, W.W., Brown, C.L., Sullivan, C.V., and Bern, H.A.**, Fish and Amphibian Models for Developmental Endocrinology. *J. Exp. Zool.* 4, 90, 1990.

39. **Young, G.**, Cortisol Secretion in Vitro by the Interrenal of Coho Salmon (*Onchorhynchus Kisutch*) During Smoltification: Relationship With Plasma Thyroxine and Plasma Cortisol., *Gen. Comp. Endocrinol.*, 63, 191, 1986.

40. **Young, G. and Lin, R.J.**, Response of the Interrenal to Adrenocorticotropic Hormone After Short-Term Thyroxine Treatment of Coho Salmon (*Onchorhynchus Kisutch*). *J. Exp. Zool.*, 245, 53, 1988.

41. **Beyer, C.a.H.J.B.**, Androgens Stimulate the Morphological Maturation of Embryonic Hypothalamic Aromatase-Immunoreactive Neurons in the Mouse. *Dev. Brain Res.*, 98, 74, 1997.

42. **Lephart, E.D.**, A Review of Brain Aromatase Cytochrome P450., *Brain Res. Rev.*, 1, 1996.

43. **Naftolin, F., Horvath, T.L., Jakab, R.L., Leranth, C., Harada, N., and Balthazart, J.**, Aromatase Immunoreactivity in Axon Terminals of the Vertebrate Brain., *Neuroendocrinology*, 63, 149, 1996.

44. **Hayes, T.B. and Licht, P.**, Gonadal Involvement in Size Sex Dimorphism in the African Bullfrog (*Pyxicephalus Adspersus*)., *J. Exp. Zool.* 264, 130, 1992.

45. **Hews, D.K. and Moore, M.C.**, Influence of Androgens on Differentiation of Secondary Sex Characters in Tree Lizards, *Urosaurus Ornatus.*, *Gen. Comp. Endocr.*, 97, 86, 1995.

46. **Kanamadi, R.D. and Saidapur, S.K.**, Effect of Testosterone on Spermatogenesis Leydig Cells and Thumb Pads of the Frog *Rana Cyanophlyctis*, *J. Karnatak Univ. Sci.*, 31, 157, 1993.

47. **Pawar, V.G. and Pancharatna, K.**, Estradiol-17-â Induced Oviductal Growth in the Skipper Frog *Rana Cyanophlyctis*, *J. Adv. Zool*, 16, 107, 1995.

48. **Rabelo, E.M. and Tata, J.R.**, Thyroid Hormone Potentiates Estrogen Activation of Vitellogenin Genes and Autoinduction of Estrogen Receptor in Adult *Xenopus* Hepatocytes., *Mol. Cell. Endocrinol.*, 96, 37, 1993.

49. **Rabelo, E.M.L., Baker, B.S., and Tata, J.R.**, Interplay Between Thyroid Hormone and Estrogen in Modulating Expression of Their Receptor and Vitellogenin Genes During *Xenopus* Metamorphosis., *Mech. Develop.*, 45, 49, 1994.

50. **Van den Hurk, R., Richter, C.J.J., and Janssen-Dommerholt, J.**, Effects of 17á-Methyltestosterone and 11â-Hydroxyandrostenedione on Gonad Differentiation in the African Catfish, *Clarias Gariepinus*,

Aquaculture, 83, 179, 1989.

51. **Jansen, H.T., Cooke, P.S., Porcelli, J., Liu, T.C., and Hansen, L.G.**, Estrogenic and Antiestrogenic Actions of PCBs in the Female Rat: in Vitro and in Vivo Studies., *Reprod. Toxicol.,* 7, 237, 1993.

52. **Faber, K.A., Basham, K., and Hughes, C.L.**, The Effect of Neonatal Exposure to DES and O,p'-DDT on Pituitary Responsiveness to GnRH in Adult Castrated Rats., *Reprod. Toxicol.,* 5, 363, 1991.

53. **Moore, R.W., Jefcoate, C.R., and Peterson, R.E.**, 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin Inhibits Steroidogenesis in the Rat Testis by Inhibiting the Mobilization of Cholesterol to Cytochrome P450scc. *Toxicol. Appl. Pharm.* 109, 85, 1991.

54. **Mukherjee, D., Guha D., Kumar V., and Chakrabarty S.**, Impairment of Steroidogenesis and Reproduction in Sexually Mature *Cyprinus Carpio* by Phenol and Sulfide Under Laboratory Conditions, *Aquatic Toxicology,* 21, 29, 1991.

55. **Singh, P.**, Impact of Malathion and Â-BHC on Lipid Metabolism in the Freshwater Female Catfish, Heteropneustes Fossilis , *Ecotoxicology and Environmental Safety,* 23, 22, 1992.

56. **McMaster, M.E., van der Kraak, G.J., and Munkittrick, K.R.,** Biochemical Basis for Hormonal Dysfunctions of Fish Exposed to Organic Wastes [Abstract], in *Proceedings of the 38th Conference of the International Association of Great Lakes Research.*, Eds., International Association for Great Lakes Research, Ann Arbor (MI), 1995, 30.

57. **Kapur, K., Kamaldeep, K., and Toor, H.**, The Effect of Fenitrothion on Reproduction of a Teleost Fish, Cyprinus Carpio Communis (Linn.): a Biochemical Study , *Bulletin of Environmental Contamination and Toxicology.*, 20, 438, 1978.

58. **Kirubagaran, R. and Joy, K.P.**, Toxic Effects of Mercuric-Chloride, Methylmercuric Chloride, and Emisan-6 (an Organic Mercurial Fungicide) on Ovarian Recrudescence in the Catfish Clarias-Batrachus (L), *Bulletin of Environmental Contamination and Toxicology,* 41, 902, 1988.

59. **Bagchi, P., Chatterjee, S., Ray, A., and Deb, C.**, Effect of Quinalphos, Organophosphorous Insecticide, on Testicular Steroidogenesis in Fish, Clarias Batrachus , *Bulletin of Environmental Contamination and Toxicology,* 44, 871, 1990.

60. **Foster, P., Thomas, L., Cook, M., and Walters, D.**, Effect of Di-n-Pentyl Phthalate Treatment on Testicular Steroidogenic Enzymes and Cytochrome P-450 in the Rat, *Toxicology Letters,* 15, 265, 1983.

61. **Goldstein, J.A. and Safe, S.**, Mechanism of Action and Structure-Activity Relationships for the Chlorinated Dibenzo-p-Dioxins and Related Compounds., in *Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products,* Kimbrough, R.D. and Jensen, A.A., Eds., Elsevier Science Publishers, Amsterdam, 1989, 239.

62. **Sivarajah, K., Franklin, C.S., and Williams, W.P.**, The Effects of Polychlorinated Biphenyls on Plasma Steroid Levels and Hepatic Microsomal Enzymes in Fish, *Journal of Fish Biology,* 13, 401, 1978.

63. **Ankley, G.T., Blazer, V.S., Reinert, R.E., and Agosin, M.**, Effects of Aroclor-1254 on Cytochrome-P-450-Dependent Monooxygenase, Glutathione-S-Transferase, and Udp- Glucuronosyltransferase Activities in Channel Catfish Liver, *Aquatic Toxicology,* 9, 91, 1986.

64. **Goldzieher, J.W.**, Are Low-Dose Oral-Contraceptives Safer and Better, *American Journal of Obstetrics and Gynecology,* 171, 587, 1994.

65. **Darnerud, P.O., Morse, D., Klassonwehler, E., and Brouwer, A.**, Binding of a 3,3',4,4'-Tetrachlorobiphenyl (Cb-77) Metabolite to Fetal Transthyretin and Effects on Fetal Thyroid Hormone Levels in Mice, *Toxicology,* 106, 105, 1996.

66. **Darnerud, P.O., Sinjari, T., and Jonsson, C.J.**, Foetal Uptake of Coplanar Polychlorinated Biphenyl (Pcb) Congeners in Mice, *Pharmacology & Toxicology,* 78, 187, 1996.

67. **Barter, R.A. and Klaassen, C.D.**, Reduction of Thyroid Hormone Levels and Alteration of Thyroid Function by Four Representative UDP-Glucuronosyltransferase Inducers in Rats. *Toxicol. Appl. Pharm.* 128, 9, 1994.

68. **Seo, B.-W., Li, M.-H., Hansen, L.G., Moore, R.W., Peterson, R.E., and Schantz, S.L.**, Effects of Gestational and Lactational Exposure to Coplanar Polychlorinated Biphenyl (PCB) Congeners or 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) on Thyroid Hormone Concentrations in Weanling Rats. *Toxicol. Letters,* 78, 253, 1995.

69. **Van Birgelen, A.P.J.M., Smit, E.A., Kampen, I.M., Groeneveld, C.N., Fase, K.M., Van Der Kolk, J., Poiger, H., Van Den Berg, M., Koeman, J.H., and Brouwer, A.**, Subchronic Effects of 2,3,7,8-TCDD or PCBs on Thyroid Hormone Metabolism: Use in Risk Assessment, *Eur. J. Pharmacol. Environ. Toxicol. Pharmacol. Sect,* 5, 77, 1995.

70. **Rosner, W.**, The Functions of Corticosteroid-Binding Globulin and Sex Hormone-Binding Globulin: Recent Advances, *Endocrine Reviews,* 11, 80, 1990.

71. **Arnold, Steven F., Diane M. Klotz, Bridgette M. Collins, Peter M. Vonier, Louis J. Jr. Guillette, and John A. McLachlan**. "Synergistic Activation of Estrogen Receptor With Combinations of Environmental Chemicals.", 1489-91. 1996.

72. **Anderson, M.J., Olsen, H., Matsumura, F., and Hinton, D.E.**, In Vivo Modulation of 17-â-Estradiol-Induced Vitellogenin Synthesis and Estrogen Receptor in Rainbow Trout *(Oncorhynchus Mykiss).* *Toxicol. Appl. Pharm.* 137, 210, 1996.

73. **Anderson, M.J., Miller, M.R., and Hinton, D.E.**, In Vitro Modulation of 17-â-Estradiol-Induced Vitellogenin Synthesis: Effects of Cytochrome P4501A1 Inducing Compounds on Rainbow Trout *(Oncorhynchus Mykiss)* Liver Cells., *Aquat.Toxicol.,* 34, 327, 1996.

74. **Flouriot, G., Pakdel, F., Ducouret, B., and Valotaire, Y.**, Influence of Xenobiotics on Rainbow Trout Liver Estrogen Receptor and Vitellogenin Gene Expression., *J. Mol. Endocrinol.,* 15, 143, 1995.

75. **Danzo, B.J.**, Environmental Xenobiotics May Disrupt Normal Endocrine Function by Interfering With the Binding of Physiological Ligands to Steroid Receptors and Binding Proteins., *Environ. Health Persp.* 105, 294, 1997.

76. **Gray, L.E.Jr., Kelce, W.R., Wiese, T., Tyl, R., Gaido, K., Cook, J., Klinefelder, G., Desaulniers, D., Wilson, E., Zacharewski, T., Waller, C., Foster, P., Lasky, J., Reel, J.G.J., Laws, S., McLachlan, J., Breslin, W., Cooper, R., DiGiulio, R., Johnson, R., Purdy, R., Mihaich, E., Safe, S., Sonnenschein, C., Weshons, W., Miller, R., McMaster, S., and Colborn. T.** Endocrine Screening Methods. Workshop Report: Detection of Estrogenic and Androgenic Hormonal and Antihormonal Activity for Chemicals That Act Via Receptor or Steroidogenic Enzyme Mechanisms., *Reprod. Toxicol.,* 11, 719, 1997.

77. **Kelce, W.R., Monosson, E., Gamcsik, M.P., Laws, S.C., and Gray, L.E.**, Environmental Hormone

Disruptors: Evidence That Vinclozolin Developmental Toxicity Is Mediated by Antiandrogenic Metabolites., *Toxicol. Appl. Pharm.* 126, 276, 1994.

78. **Hoffman, E.C., Reyes, H., Chu, F.-F., Sander, F., Conley, L.H., Brooks, B.A., and Hankinson, O.**, Cloning of a Factor Required for Activity of the Ah (Dioxin) Receptor, *Science,* 252, 954, 1991.

79. **Reyes, H., Reisz-Porszasz, S., and Hankinson, O.**, Identification of the Ah Receptor Nuclear Translocator Protein (Arnt) As a Component of the DNA Binding Form of the Ah Receptor, *Science,* 256, 1193, 1992.

80. **DeVito, M.J., Thomas, T., Martin, E., Umbreit, T.H., and Gallo, M.A.**, Antiestrogenic Action of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin: Tissue-Specific Regulation of Estrogen Receptor in CDI Mice, *Toxicol. Appl. Pharmacol.*, 113, 284, 1992.

81. **Kharat, I. and Saatcioglu, F.**, Antiestrogenic Effects of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin Are Mediated by Direct Transcriptional Interference With the Liganded Estrogen Receptor - Cross-Talk Between Aryl Hydrocarbon- and Estrogen-Mediated Signaling, *Journal of Biological Chemistrry,* 271, 10533, 1996.

82. **Zacharewski, T., Harris, M., and Safe, S.**, Evidence for the Mechanism of Action of the 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin-Mediated Decrease of Nuclear Estrogen Receptor Levels in Wild-Type and Mutant Mouse Hepa 1c1c7 Cells, *Biochemical Pharmacology,* 41, 1931, 1991.

83. **Tian, Y., Ke, S., Thomas, T., Meeker, R.J., and Gallo, M.A.**, Transcriptional Suppression of Estrogen Receptor Gene Expression by 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD), *Journal of Steroid Biochemistry and Molecular Biology,* 67, 17, 1998.

84. **McLachlan, J.A.**, Functional Toxicology: A New Approach to Detect Biologically Active Xenobiotics., *Environ. Health Persp.* 101, 386, 1993.

85. **Hwang, K.J., Carlson, K.E., and Anstead, G.M.a.K.J.A.**, Donor-Acceptor Tetrahydrochrysenes, Inherently Fluorescent, High Affinity, Ligands for the Estrogen Receptor: Binding and Fluorescence Characteristics and Fluorometric Assay of Receptor., *Biochemistry,* 31, 11536, 1992.

86. **Zysk, J.R., Johnson, B., Ozenberger, B.A., Bingham, B., and Gorski, J.**, Selective Uptake of Estrogenic Compounds by *Saccharomyces Cerevesiae*: a Mechanism for Antiestrogen Resistance in Yeast Expressing the Mammalian Estrogen Receptor., *Endocrinology,* 136, 1323, 1995.

87. **Miksicek, R.J.**, Commonly Occurring Plant Flavonoids Have Estrogenic Activity., *Mol. Pharmacol.*, 44, 37, 1993.

88. **Brooks, S.C., Locke, E.R., and Soule, H.D.**, Estrogen Receptor in a Human Cell Line (MCF-7) From Breast Carcinoma., *J. Biol. Chem.*, 248, 6251, 1973.

89. **Soto, A.M., Chung, K.L., and Sonnenschein, C.**, The Pesticides Endosulfan, Toxaphene and Dieldrin Have Estrogenic Effects in Human Estrogen-Sensitive Cells. *Environ. Health Persp.* 102, 380, 1994.

90. **Soto, A.M., Justicia, H., Wray, J.W., and Sonnenschein, C.**, P-Nonyl Phenol: an Estrogenic Xenobiotic Released From "Modified" Polystyrene. *Environ. Health Persp.* 92, 167, 1991.

91. **Soto, A.M., Lin, T.M., Justicia, H., Silvia, R.M., and Sonnennschein, C.,** An "in Culture" Bioassay to Assess the Estrogenicity of Xenobiotics (E-Screen)., in., Eds., Princeton Scientific Publishing, Princeton, NJ, USA, 1992, 295.

92. **Soto, A.M., Sonnenschein, C., Chung, K.L., Fernandez, M.F., Olea, N., and Serrano, F.O.**, The E-SCREEN Assay As a Tool to Identify Estrogens: an Update on Estrogenic Environmental Pollutants. *Environ. Health Persp.* 103, 113, 1995.

93. **Soto, A.M. and Sonnenshein, C.**, The Role of Estrogens on the Proliferation of Human Breast Tumor Cells (MCF-7)., *J. of Steroid Biochem.* 23, 87, 1985.

94. **Welshons, W.V., Rottinghaus, G.E., Nonneman, D.J., Dolan-Timpe, M., and Ross, P.F.**, A Sensitive Bioassay for Detection of Dietary Estrogens in Animal Feeds. *J. Vet. Diagn. Invest.* 2, 268, 1990.

95. **White, R., Jobling, S., Hoare, S.A., Sumpter, J.P., and Parker, M.G.**, Environmentally Persistent Alkylphenolic Compounds Are Estrogenic. *Endocrinology,* 135, 175, 1994.

96. **Olea, N., Pulgar, R., Perez, P., Olea-Serrano, F., Rivas, A., Novillo-Fertrell, A., Pedraza, V., Soto, A.M., and Sonnenschein, C.**, Estrogenicity of Resin-Based Composites and Sealants Used in Dentistry., *Environ. Health Persp.* 104, 298, 1996.

97. **Folmar, L.D., Denslow, N.D., Wallace, R.A., LaFleur, G., Gross, T.S., Bonomelli, S., and Sullivan, C.V.**, A Highly Conserved N-Terminal Sequence for Teleost Vitellogenin With Potential Value to the Biochemistry, Molecular Biology and Pathology of Vitellogenesis., *J. Fish Biol.* 46, 255, 1995.

98. **Denslow, N.D., Chow, M., Kroll, K., Wieser, C., Wiebe, J., Johnson, B., Shoeb, T., and Gross, T.S.,** Determination of Baseline Seasonal Information on Vitellogenin Production in Female and Male Largemouth Bass Collected From Lakes in Central Florida. in *18th Annual Meeting SETAC.*, Eds., 1997

99. **Zacharewski, T.**, In Vitro Bioassays for Assessing Estrogenic Substances., *Environ. Sci. Technol.* 31, 613, 1997.

100. **Pons, M., Gagne, D., Nicolas, J.C., and Mehtali, M.**, A New Cellular Model of Response to Estrogens: a Bioluminescent Test to Characterize (Anti) Estrogen Molecules, *BioTechniques,* 456, 1990.

101. **Gaido, K.W., Leonard, L.S., Lovell, S., Gould, J.C., Babai, D., Portier, C.J., and McDonnell, D.P.**, Evaluation of Chemicals With Endocrine Modulation Activity in a Yeast-Based Steroid Hormone Receptor Gene Transcription Assay., *Toxicol. Appl. Pharm.* 143, 205, 1997.

102. **Ramamoorthy, K., Wang, F., Chen, I.C., Norris, J.D., McDonnell, D.P., Leonard, L.S., Gaido, K.W., Bocchinfuso, W.P., Korach, K.S., and Safe, S.**, Estrogenic Activity of a Dieldrin/Toxaphene Mixture in the Mouse Uterus, MCF-7 Human Breast Cancer Cells, and Yeast-Based Estrogen Receptor Assays: No Apparent Synergism., *Endocrinology,* 138, 1520, 1997.

103. **Demirpence, E., Pons, M., Balaguer, P., and Gagne, D.**, Study of an Antiestrogenic Effect of Retinoic Acid in MCF-7 Cells. *Biochem. Bioph. Res. Co.,* 183, 100, 1992.

104. **Stegeman, J.J., Brouwer, M., Di Giulio, R.T., Förlin, L., Fowler, B.A., Sanders, B.M., and Van Veld, P.A.,** Molecular Responses to Environmental Contamination: Enzyme and Protein Systems As Indicators of Chemical Exposure and Effect, in *Biomarkers: Biochemical, Physiological, and Histological Markers of Anthropogenic Stress. Proceedings of the 8th Pellston Workshop,* Huggett, R.J., Kimerle, R.A., Mehrle, P.M., and Bergman, H.L., Eds., Lewis Publishers, Chelsea (MI), 1992

105. **Sumpter, J.P. and Jobling, S.**, Vitellogenesis As a Biomarker for Estrogenic Contamination of the Aquatic Environment., *Environ. Health Persp.* 103, 173, 1995.

106. **Down, N.E., Peter, R.E., and Leatherland, J.F.**, Seasonal Changes in Serum Gonadotropin, Testosterone, 11-Ketotestosterone and Estradiol Levels and Their Relation to Tumor Burden in Gonadal Tumor Bearing Carp in the Great Lakes., *Gen. Comp. Endocr.*, 77, 192, 1990.

107. **Goodbred, S. L., R. L. Gilliom, T. S. Gross, N. D. Denslow, W. Bryant**, and **T. R. Schoeb**. *Reconnaisance of 17- Â-Estradiol, 11-Ketotestosterone Vitellogenin and Gonad Histopathology in Common Carp of United States Streams: Potential for Contaminant-Induced Endocrine Disruption.*, 96-627. USGS, Sacramento, CA, USA., 1997.

108. **Gross, T.S., Wiebe, J., Wieser, C., Shrestha, S., Reiskind, M., Denslow, N., Johnson, W.E., and Stout, R.**, Endocrine-Disrupting Effects of Pesticides in Largemouth Bass: An Examination of Potential Synergistic Effects, in *18th Annual Meeting SETAC.*, Eds., 1997

109. **Gross, T.S., Wiebe, J.J., Wieser, C.M., Ruessler, D.S., and Aikens, K.,** An Evaluation of Methyl Mercury As an Endocrine Disruptor in
Largemouth Bass. in *Proceedings: 6th International Symposium on Metal Ions in Biology and Medicine..*, Eds., 2000

110. **Hontela, A., Dumont, P., Duclos, D., and Fortin, R.**, Endocrine and Metabolic Dysfunction in Yellow Perch Exposed to Organic Contaminants and Heavy Metals in the St. Lawrence River., *Environ.Toxicol. Chem.* 14, 725, 1995.

111. **Leatherland, J.F.,** Endocrine and Reproductive Function in Great Lakes Salmon, in., Eds., Princeton Sci. Publ. Co., Inc., Princeton, NJ, USA, 1992, 129.

112. **Sanglang, B. and Freeman, H.C.**, Effect of Sublethal Cadmium on Maturation and Testosterone and 11-Ketotestosterone Production in Vivo in Brook Trout. *Biol. Reprod.*, 11, 429, 1974.

113. **Copeland, P.A. and Thomas, P.**, The Measurement of Plasma Vitellogenin Levels in a Marine Teleost, the Spotted Seatrout (*Cynoscion Nebulosus*) by Homologous Radioimmunoassay. *Comp. Biochem. Physiol.*, 91, 17, 1988.

114. **Denison, M.S., Chambers, J.E., and Yarbrough, J.D.**, Persistent Vitellogenin-Like Protein and Binding of DDT in the Serum of Insecticide-Resistant Mosquitofish (*Gambusia Affinis*). *Comp. Biochem. Phys. C:* 69, 109, 1981.

115. **Carnevali, O. and Belveldere, P.**, Comparative Studies of Fish, Amphibian and Reptilian Vitellogenins. *J. Exp. Zool.* 259, 18, 1991.

116. **Crews, D., Bergeron, J.M., and McLachlan, J.A.**, The Role of Estrogen in Turtle Sex Determination and the Effect of PCBs., *Environ. Health Persp.* 103, 73, 1995.

117. **Davis, W.P. and Bortone, S.A.,** Effects of Kraft Mill Effluents on the Sexuality of Fishes: an Environmental Early Warning? in., Eds., Princeton Sci. Publ. Co., Inc., Princeton, NJ, USA, 1992.

118. **Gimeno S., Gerritsen, A., Bowmer, T., and Koeman, H.**, Feminization of Male Carp. *Nature,* 384, 221,

1996.

119. **Miles-Richardson, S.R., Fitzgerald, S.D., Render, J., Barbee, S., Giesy, J.P., and Kramer, V.J.**, Effects of Waterborne Exposure of 17-â-Estradiol on Secondary Sex Characteristics and Gonads of Fathead Minnow (*Pimephales Promelas*). 1998.

120. **Munkittrick, M.R., Van Der Kraak, G.J., McMaster, M.E., and Port, C.B.**, Response of Hepatic MFO Activity and Plasma Sex Steroids to Secondary Treatment of Bleached Kraft Mill Effluent and Mill Shutdown., *Environ.Toxicol. Chem.* 11, 1427, 1992.

121. **Gross, T.S., Guillette, L.J., Percival, H.F., Masson, G.R., Matter, J.M., and Woodward, A.R.**, Contaminant-Induced Reproductive Anomalies in Florida Alligators. *Comp. Pathol. Bull.*, 26, 1, 1994.

122. **Sepúlveda, M.S., Ruessler, D.S., Denslow, N.D., Holm, S.E., and Gross, T.S.**, Effects of Paper Mill Effluents on the Reproductive Success of Florida Largemouth Bass, in *Proceedings of the International Conferance on Pulp and Papermill Research.*, Eds., 2000

123. **Sepúlveda, M.S., Wiebe, J.J., Harvey, A., Basto, J., Ruessler, D.S., Roldan, E., and Gross, T.S.**, An Evaluation of Environmental Contaminants and Developmental Toxicity for the American Alligator in Central Florida., in *2001 Society of Toxicology Annual Meeting.*, Eds., 2001

124. **Giroux, D.** "Correlative Evaluation of Chlorinated Hydrocarbon Residues and Reproductive Anomalies in Alligators." University of Florida, 1997.

125. **Guillette, L.J.Jr., Gross, T.S., Masson, G.R., Matter, J.M., Percival, H.F., and Woodward, A.R.**, Developmental Abnormalities of the Gonad and Abnormal Sex Hormone Concentrations in Juvenile Alligators From Contaminated and Control Lakes in Florida, *Environmental Health Perspectives*, 102, 680, 1994.

126. **Masson, G. R.** " Environmental Influences on Reproductive Potential, Clutch Viability and Embryonic Mortality of the American Alligator in Florida." University of Florida, 1995.

127. **Hose, J.E., Cross, J.N., Smith, S.G.S., and Diehl, D.**, Reproductive Impairment in a Fish Inhabiting a Contaminated Coastal Environment Off of Southern California. *Environ. Pollut.*, 57, 139, 1989.

128. **Guillette, L.J., Gross, T.S., Masson, G.R., Matter, J.M., Percival, H.F., and Woodward, A.R.**, Developmental Abnormalities of the Gonad of Juvenile Alligators From Contaminated and Control Lakes in Florida., *Environ. Health Persp.* 102, 680, 1994.

129. **Gross, D. A.** "Thymus, Spleen and Bone Marrow Hypoplasia and Decreased Antibody Responses in Hatchling Lake Apopka Alligators. " University of Florida, 1997.

130. **Percival, H.F., Rice, K., Woodward, A., Jennings, M., Masson, G., and Abercombie, C.**, Depressed Alligator Clutch Viability on Lake Apopka, Florida, in *Fifth Annual Lake Management Scoiety Symposium, World Meeting Number 942 5020.*, Eds., 1994

131. **Guillette, L.J.Jr., Gross, T.S., Masson, G.R., Matter, J.M., Percival, H.F., and Woodward, A.R.**, Developmental Abnormalities of the Gonad and Abnormal Sex Hormone Concentrations in Juvenile Alligators From Contaminated and Control Lakes in Florida, *Environmental Health Perspectives*, 102, 680, 1994.

132. **Gross, T.S., Guillette, L.J., Percival, H.F., Masson, G.R., Matter, J.M., and Woodward, A.R.**, Contaminant Induced Reproductive Anomalies in Florida Alligators, *Comparitive Pathology Bulletin,* 4, 2, 1995.

133. **Fairbrother, A., Ankley, G.T., Birnbaum, L.S., Bradbury, S.P., Francis, B., Gray, L.E., Hinton, D., Johnson, L.L., Peterson, R.E., and Van Der Kraak, G.**, Reprodutive and Developmental Toxicology of Contaminants in Oviparous Animals, in *Reproductive and Developmental Effects of Contaminants in Oviparous Vertebrates. SETAC Pellston Workshop on Reproductive and Developmental Effects of Contaminants in Oviparous Vertebrates; Fairmont Hot Springs, Anaconda, Montana,* Di Giulio, R.T. and Tillitt, D.E., Eds., SETAC Press, Pensacola, Fl, 1999.

134. **Hoffman, D.J.**, Embryotoxicity and Teratogenicity of Environmental Contaminants to Bird Eggs, *Reviews of Environmental Contamination and Toxicology,* 115, 39, 1990.

135. **Fry, D.M.**, Reproductive Effects in Birds Exposed to Pesticides and Industrial Chemicals, *Environmental Health Perspectives,* 103, 165, 1995.

136. **Fry, D.M., Swenson, J., Addiego, L.A., Grau, C.R., and Kang, A.**, Reduced Reproduction of Wedge-Tailed Shearwaters Exposed to Weathered Santa-Barbara Crude-Oil, *Archives of Environmental Contamination and Toxicology,* 15, 453, 1986.

137. **Trivelpiece, W.Z., Butler, R.G., Miller, D.S., and Peakall, D.B.**, Reduced Survival of Chicks of Oil-Dosed Adult Leachs Storm- Petrels, *Condor,* 86, 81, 1984.

138. **Oakley, K.L. and Kuletz, K.**, Population, Reproduction, and Foraging of Pigeon Guillemots at Naked Island, Alaska, Before and After the *Exxon Valdez* Oil Spill, *American Fisheries Society Symposium,* 18, 759, 1996.

139. **Sharpe, B.E., Cody, M., and Turner R.**, Effects of the *Exxon Valdez* Oil Spill on the Black Oystercatcher, *American Fisheries Society Symposium,* 18, 748, 1996.

140. **Cavanaugh, K.P. and Holmes, W.N.**, Effects of Ingested Petroleum on Plasma-Levels of Ovarian- Steroid Hormones in Photostimulated Mallard Ducks, *Archives of Environmental Contamination and Toxicology,* 11, 503, 1982.

141. **Thomas, P.**, Reproductive Endocrine Function in Female Atlantic Croaker Exposed to Pollutants, *Marine Environmental Research,* 24, 179, 1988.

142. **Thomas, P.**, Teleost Model for Studying the Effects of Chemicals on Female Reproductive Endocrine Function, *Journal of Experimental Zoology,* 126, 1990.

143. **Thomas, P. and Smith, J.**, Binding of Xenobiotics to the Estrogen Receptor of Spotted Seatrout: a Screening Assay for Potential Estrogenic Effects, *Marine Environmental Research,* 35, 147, 1993.

144. **Singh, H.**, Interaction of Xenobiotics With Reproductive Endocrine Functions in a Protogynous Teleost, *Monopterus Albus, Marine Environmental Research,* 28, 285, 1989.

145. **Johnson, L.L., Casillas, E., Collier, T.K., McCain, B.B., and Varanasi, U.**, Contaminant Effects on Ovarian Development in English Sole (*Parophyrs Vetulus*) From Puget Sound, Washington, *Canadian Journal of Fisheries and Aquatic Sciences*, 45, 2133, 1988.

146. **Johnson, L., Casillas, E., Sol, S., Collier, T., Stein, J., and Varanasi, U.**, Contaminant Effects on Reproductive Success in Selected Benthic Fish, *Marine Environmental Research*, 35, 165, 1993.

147. **Stott, G.G., Haensly, W.E., Neff, J.M., and Sharp, J.R.**, Histopathologic Survey of Ovaries of Plaice, Pleuronectes- Platessa L, From Aber Wrach and Aber Benoit, Brittany, France - Long-Term Effects of the Amoco Cadiz Crude-Oil Spill, *Journal of Fish Diseases*, 6, 429, 1983.

148. **McMaster, M.E., Munkittrick, K.R., Luxon, P.L., and van der Kraak, G.J.**, Impact of Low-Level Sampling Stress on Interpretation of Physiological Responses of White Sucker Exposed to Effluent From a Bleached Kraft Pulp Mill, *Ecotoxicology and Environmental Safety*, 27, 251, 1994.

149. **Slooff, W., Dezwart, D., and Vandekerkhoff, J.F.J.**, Monitoring the Rivers Rhine and Meuse in the Netherlands for Toxicity, *Aquatic Toxicology*, 4, 189, 1983.

150. **Stott, G.G., Mcarthur, N.H., Tarpley, R., Sis, R.F., and Jacobs, V.**, Histopathological Survey of Male Gonads of Fish From Petroleum Production and Control Sites in the Gulf of Mexico, *Journal of Fish Biology*, 17, 593, 1980.

151. **Stott, G.G., Mcarthur, N.H., Tarpley, R., Jacobs, V., and Sis, R.F.**, Histopathologic Survey of Ovaries of Fish From Petroleum Production and Control Sites in the Gulf of Mexico, *Journal of Fish Biology*, 18, 261, 1981.

152. **Anderson, M.J., Miller, M.R., and Hinton, D.E.**, *In Vitro* Modulation of 17ß-Estradiol-Induced Vitellogenin Synthesis: Effects of Cytochrome P4501A1 Inducing Compounds on Rainbow Trout (*Oncorhynchus Mykiss*) Liver Cells, *Aquatic Toxicology*, 34, 327, 1996.

153. **Safe, S., Astroff, B., Harris, M., Zacharewski, T., Dickerson, R., Romkes, M., and Biegel, L.**, 2,3,7,8-Tetrachlorodibenzo-o-Dioxin (TCDD) and Related Compounds As Antiestrogens: Characterization and Mechanism of Action, *Pharmacology and Toxicology*, 69, 400, 1991.

154. **Rocha Moneiro, P.R.**, Polycyclic Aromatic Hydrocarbons Inhibit in Vitro Ovarian Steroidogenesis in the Flounder (*Platichthys Flesus* L.), *Aquatic Toxicology*, 48, 549, 2000.

155. **Mackay, D., Sang, S., Vlahos, P., Gobas, F., Diamond, M., and Dolan, D.**, A Rate Constant Model of Chemical Dynamics in a Lake Ecosystem; PCBs in Lake Ontario, *Journal of Great Lakes Research*, 20, 625, 1994.

156. **Janz, D.M., Metcalfe, T.L., Metcalfe, C.D., and Haffner, G.D.**, Relative Concentrations of Cytochrome P450-Active Organochlorine Compounds in Liver and Muscle of Rainbow Trout From Lake Ontario, *Journal of Great Lakes Research*, 18, 759, 1992.

157. **Safe, S.H.**, Polychlorinated-Biphenyls (Pcbs) - Environmental-Impact, Biochemical and Toxic Responses, and Implications for Risk Assessment, *Critical Reviews in Toxicology*, 24, 87, 1994.

158. **Wren, C.D.**, Cause-Effect Linkages Between Chemicals and Populations of Mink (Mustela-Vison) and Otter (Lutra-Canadensis) in the Great-Lakes Basin, *Journal of Toxicology and Environmental Health,* 33, 549, 1991.

159. **Foley, R.E., Jackling, S.J., Sloan, R.J., and Brown, M.K.**, Organochlorine and Mercury Residues in Wild Mink and Otter - Comparison With Fish, *Environmental Toxicology and Chemistry,* 7, 363, 1988.

160. **Platonow, N.S. and Karstad, L.H.**, Dietary Effects of Polychlorinated Biphenyls on Mink, *Canadian Journal of Comparative Medicine-Revue Canadienne De Medecine Comparee,* 37, 391, 1973.

161. **Hornshaw, T.C., Aulerich, R.J., and Johnson, H.E.**, Feeding Great-Lakes Fish to Mink - Effects on Mink and Accumulation and Elimination of Pcbs by Mink, *Journal of Toxicology and Environmental Health,* 11, 933, 1983.

162. **Clark, D.R.Jr. and Lamont, T.G.**, Organochlorine Residues and Reproduction in Big Brown Bat, *Journal of Wildlife Management,* 40, 249, 1976.

163. **Clark, D.R.Jr. and Krynitsky, A.,** Organochlorine Residues and Reproduction in the Little Brown Bat, in *Pestic. Monit. J..*, Eds., 1978

164. **Clark, D.R. and Stafford, C.J.**, Effects of Dde and Pcb (Aroclor 1260) on Experimentally Poisoned Female Little Brown Bats (Myotis-Lucifugus) - Lethal Brain Concentrations, *Journal of Toxicology and Environmental Health,* 7, 925, 1981.

165. **Helle, E., Olsson M., and Jensen S.**, PCB Levels Correlated With Pathological Changes in Seal Uteri, *Ambio,* 5, 261, 1976.

166. **Perttila, M., Stenman, O., Pyysalo, H., and Wickstrom, K.**, Heavy-Metals and Organochlorine Compounds in Seals in the Gulf of Finland, *Marine Environmental Research,* 18, 43, 1986.

167. **DeLong, R.L., Gilmartin W.G., and Simpson J.G.**, Premature Births in California Sea Lions: Association With High Organochlorine Pollutant Residue Levels, *Science,* 181, 1168, 1973.

168. **Martineau, D., Beland, P., Desjardins, C., and Lagace, A.**, Levels of Organochlorine Chemicals in Tissues of Beluga Whales, *Archives of Environmental Contamination and Toxicology,* 16, 137, 1987.

169. **Bergman, A., Olsson, M., and Reiland, S.**, Skull-Bone Lesions in the Baltic Grey Seal (*Halichoerus Grypus*), *Ambio,* 21, 517, 1992.

170. **Mortensen, P., Bignert, A., and Olsson, M.**, Prevalence of Skull Lesions in Harbor Seals (*Phoca Vitulina*) in Swedish and Danish Museum Collections: 1835-1988, *Ambio,* 21, 520, 1992.

171. **Casteel, S.W., Cowart, R.P., Weis, C.P., Henningsen, G.M., Hoffman, E., Brattin, W.J., Guzman, R.E., Starost, M.F., Payne, J.T., Stockham, S.L., Becker, S.V., Drexler, J.W., and Turk, J.R.**, Bioavailability of Lead to Juvenile Swine Dosed With Soil From the Smuggler Mountain NPL Site of Aspen, Colorado, *Fundamental and Applied Toxicology,* 36, 177, 1997.

172. **Reijnders, P.J.H.**, Reproductive Failure in Common Seals Feeding on Fish From Polluted Coastal Waters, *Nature,* 324, 456-&, 1986.

173. **Kamrin, M.A. and Ringer, R.K.,** Toxicological Implications of PCB Residues in Mammals, in *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations,* Beyer, W.N., Heinz, G.H., and Redmon-Norwood, A.W., Eds., Lewis Publishers, Boca Raton, Fl, 1996, 153.

174. **Bowerman, W.W., Giesy, J.P., Best, D.A., and Kramer, V.J.**, A Review of Factors Affecting Productivity of Bald Eagles in the Great-Lakes Region - Implications for Recovery, *Environmental Health Perspectives,* 103, 51, 1995.

175. **Platonow, N.S. and Reinhart, B.S.**, Effects of Polychlorinated Biphenyls (Aroclor 1254) on Chicken Egg-Production, Fertility and Hatchability, *Canadian Journal of Comparative Medicine-Revue Canadienne De Medecine Comparee,* 37, 341, 1973.

176. **Britton, W.M. and Huston, T.M.**, Influence of Polychlorinated Biphenyls in Laying Hen, *Poultry Science,* 52, 1620, 1973.

177. **Scott, M.L.**, Effects of Pcbs, Ddt, and Mercury-Compounds in Chickens and Japanese Quail, *Federation Proceedings,* 36, 1888, 1977.

178. **Cecil, H.C., Bitman, Fries, G.F., Denton, C.A., Lillie, R.J., and Harris, S.J.**, Dietary P,P'-Ddt, O,P'Ddt or P,P'-Dde and Changes in Egg-Shell Characteristics and Pesticide Accumulation in Egg Contents and Body Fat of Caged White Leghorns, *Poultry Science,* 51, 130-&, 1972.

179. **Tumasonis, C.F., Bush, B., and Baker, F.D.**, PCB Levels in Egg Yolks Associated With Embryonic Mortality and Deformity of Hatched Chicks, *Archives of Environmental Contamination and Toxicology,* 1, 312, 1973.

180. **Peakall, D.B. and Peakall, M.L.**, Effect of a Polychlorinated Biphenyl on Reproduction of Artificially and Naturally Incubated Dove Eggs, *Journal of Applied Ecology,* 10, 863, 1973.

181. **Heinz, G.H., Hill, E.F., and Contrera, J.F.**, Dopamine and Norepinephrine Depletion in Ring Doves Fed Dde, Dieldrin, and Aroclor-1254, *Toxicology and Applied Pharmacology,* 53, 75, 1980.

182. **Haseltine, S.D. and Prouty, R.M.**, Aroclor-1242 and Reproductive Success of Adult Mallards (Anas-Platyrhynchos), *Environmental Research,* 23, 29, 1980.

183. **Custer, T.W. and Heinz, G.H.**, Reproductive Success and Nest Attentiveness of Mallard Ducks Fed Aroclor-1254, *Environmental Pollution Series a-Ecological and Biological,* 21, 313, 1980.

184. **Mclane, M.A.R. and Hughes, D.L.**, Reproductive Success of Screech Owls Fed Aroclor-1248, *Archives of Environmental Contamination and Toxicology,* 9, 661, 1980.

185. **Harris, M.P. and Osborn, D.**, Effect of a Polychlorinated Biphenyl on the Survival and Breeding of Puffins, *Journal of Applied Ecology,* 18, 471, 1981.

186. **Sileo, L., Karstad, L., Frank, R., Holdrinet, M.V.H., Addison, E., and Braun, H.E.**, Organochlorine Poisoning of Ring-Billed Gulls in Southern Ontario, *Journal of Wildlife Diseases,* 13, 313, 1977.

187. **Gilbertson, M.**, Pollutants in Breeding Herring Gulls in the Lower Great Lakes, *The Canadian Field-Naturalist,* 88, 273, 1974.

188. **Gilbertson, M. and Hale, R.**, Early Embryonic Mortality in a Herring Gull Colony in Lake Ontario, *The*

Canadian Field-Naturalist, 88, 354, 1974.

189. **Keith, J.A.**, Reproduction in a Population of Herring Gulls (*Larus Argentatus*) Contaminated by DDT, *Journal of Applied Toxicology,* 3, 57, 1966.

190. **Ludwig, J. and Tomoff, C.**, Reproductive Success and Insecticide Residues in Lake Michigan Herring Gulls, *Jack-Pine Warbler,* 44, 77, 1966.

191. **Hoffman, D.J., Rattner, B.A., Sileo, L., Docherty, D., and Kubiak, T.J.**, Embryotoxicity, Teratogenicity, and Aryl-Hydrocarbon Hydroxylase-Activity in Forsters Terns on Green-Bay, Lake- Michigan, *Environmental Research,* 42, 176, 1987.

192. **Kubiak, T.J., Harris, H.J., Smith, L.M., Schwartz, T.R., Stalling, D.L., Trick, J.A., Sileo, L., Docherty, D.E., and Erdman, T.C.**, Microcontaminants and Reproductive Impairment of the Forster's Tern on Green Bay, Lake Michigan--1983., *Archives of Environmental Contamination and Toxicology,* 18, 706, 1989.

193. **Brunstrom, B., Broman, D., and Naf, C.**, Embryotoxicity of Polycyclic Aromatic-Hydrocarbons (Pahs) in 3 Domestic Avian Species, and of Pahs and Coplanar Polychlorinated-Biphenyls (Pcbs) in the Common Eider, *Environmental Pollution,* 67, 133, 1990.

194. **Hoffman, D.J., Melancon, M.J., Eisemann, J.D., and Klein, P.N.**, Comparative Toxicity of Planar PCB Congeners by Egg Injection, *Society of Environmental Toxicology and Chemistry Abstracts,* 16, 207, 1995.

195. **Fox, G.A., Collins, B., Hayakawa, E., Weseloh, D.V., Ludwig, J.P., Kubiak, T.J., and Erdman, T.C.**, Reproductive Outcomes in Colonial Fish-Eating Birds - a Biomarker for Developmental Toxicants in Great-Lakes Food- Chains .2. Spatial Variation in the Occurrence and Prevalence of Bill Defects in Young Double-Crested Cormorants in the Great-Lakes, 1979-1987, *Journal of Great Lakes Research,* 17, 158, 1991.

196. **Yamashita, N., Tanabe, S., Ludwig, J.P., Kurita, H., Ludwig, M.E., and Tatsukawa, R.**, Embryonic Abnormalities and Organochlorine Contaminantion in Double-Creasted Cormorants (*Phalacrocorax Auritus*) and Caspian Terns (*Hydroprogne Caspia*) From the Upper Great Lakes, *Environmental Pollution,* 79, 163, 1993.

197. **Colborn, T.**, Epidemiology of Great-Lakes Bald Eagles, *Journal of Toxicology and Environmental Health,* 33, 395, 1991.

198. **Ohlendorf, H.M., Hoffman D.J., Saiki M.K., and Aldrich T.W.**, Embryonic Mortality and Abnormalities of Aquatic Birds: Apparent Impacts of Selenium From Irrigation Drainwater, *Science of the Total Environment,* 52, 49, 1986.

199. **Gilbertson, M., Kubiak, T., Ludwig, J., and Fox, G.**, Great Lakes Embryo Mortality, Edema, and Deformities Syndrome (GLEMEDS) in Colonial Fish-Eating Birds: Similarity to Chick-Edema Disease, *Journal of Toxicology and Environmental Health,* 33, 455, 1991.

200. **Heinz, G.H., Percival, H.F., and Jennings, M.L.**, Contaminants in American Alligator Eggs From Lake

Apopka, Lake Griffin, and Lake Okeechobee, Florida , *Environmental Monitoring and Assessment,* 16, 277, 1991.

201. **Guillette, L.J., Jr., Gross, T.S., Masson, G.R., Matter, J.M., Percival, H.F., and Woodward, A.R.**, Developmental Abnormalities of the Gonad and Abnormal Sex Hormone Concentrations in Juvenile Alligators From Contaminated and Control Lakes in Florida, *Environmental Health Perspectives,* 102, 680, 1994.

202. **Guillette, L.J.Jr., Pickford, D.B., Crain, D.A., Rooney, A.A., and Percival, H.F.**, Reduction in Penis Size and Plasma Testosterone Concentrations in Juvenile Alligators Living in a Contaminated Environment, *General Comparative Endocrinology,* 101, 32, 1996.

203. **Willingham, E., Rhen, T., Sakata, J.T., and Crews, D.**, Embryonic Treatment With Xenobiotics Disrupts Steroid Hormone Profiles in Hatchling Red-Eared Slider Turtles (*Trachemys Scripta Elegans*), *Environmental Health Perspectives,* 108, 329, 2000.

204. **Gutleb, A.C., Appelman, J., Bronkhorst, M.C., Van Den Berg, J.H.J., Spenkelink, A., Brouwer, A., and Murk, A.J.**, Delayed Effects of Pre- and Early-Life Time Exposure to Polychlorinated Biphenyls on Tadpoles of Two Amphibian Species (Xenopus Laevis and Rana Temporaria), *Environmental Toxicology and Pharmacology,* 8, 1, 1999.

205. **Rosenshield, M.L., Jofre, M.B., and Karasov, W.H.**, Effects of Polychlorinated Biphenyl 126 on Green Frog (Rana Clamitans) and Leopard Frog (Rana Pipiens) Hatching Success, Development, and Metamorphosis, *Environmental Toxicology and Chemistry,* 18, 2478, 1999.

206. **Johnson, L.L., Sol, S.Y., Lomax, D.P., Nelson, G.M., Sloan, C.A., and Casillas, E.**, Fecundity and Egg Weight in English Sole, Pleuronectes Vetulus, From Puget Sound, Washington: Influence of Nutritional Status and Chemical Contaminants, *Fishery Bulletin,* 95, 231, 1997.

207. **Mac, M.J. and Schwartz, T.R.**, Investigations Into the Effects of Pcb Congeners on Reproduction in Lake Trout From the Great-Lakes, *Chemosphere,* 25, 189, 1992.

208. **Mac, M.J. and Edsall, C.C.**, Environmental Contaminants and the Reproductive Success of Lake Trout in the Great-Lakes - an Epidemiologic Approach, *Journal of Toxicology and Environmental Health,* 33, 375, 1991.

209. **Johnson, L.L., Stein, J.E., Collier, T.K., Casillas, E., and Varanasi, U.**, Indicators of Reproductive Development in Prespawning Female Winter Flounder (*Pleuronectes Americanus*) From Urban and Non-Urban Estuaries in the Northeast United States, *Science of the Total Environment* , 141, 241, 1994.

210. **Ando, H. and Yano, T.**, Effects of PCB on Sexual Maturation in Female Medaka (*Oryzias Latips*), *Scientific Bulletin of the Faculty of Agriculture, Kyushu University,* 36, 79, 1982.

211. **Kidd, K., van der Kraak, G., and Munkittrick, K.**, The Effects of PCB 126 on Hepatic Mixed Function Oxygenase Activity and Steroidogenic Capacity of the Goldfish (*Carassius Auratus*), *Canadian Technical Report of Fisheries and Aquatic Sciences,* 1942, 470, 1993.

212. **Yano, T. and Matsuyama, H.**, Stimulatory Effect of PCB on the Metabolism of Sex Hormones in Carp Hepatopancreas, *Bulletin of the Japanese Society of Scientific Fisheries,* 52, 1847, 1986.

213. **Chen, T.T. and Sonstegard, R.A.**, Development of a Rapid, Sensitive and Quantitative Test for the Assessment of the Effects of Xenobiotics on Reproduction in Fish, *Marine Environmental Research,* 14, 429, 1984.

214. **Monosson, E., Fleming, W.J., and Sullivan, C.V.**, Effects of the Planar PCB 3,3',4,4'-Tetrachlorobiphenyl (TCB) on Ovarian Development, Plasma Levels of Sex Steroid Hormones and Vitellogenin, and Progeny Survival in the White Perch (*Morone Americana*), *Aquatic Toxicology,* 29, 1, 1994.

215. **Monosson, E., Hogson, R.G., Fleming, W.J., and Sullivan, C.V.**, Blood Plasma Levels of Sex Steroid Hormones and Vitellogenin in Striped Bass (Morone Saxatilis) Exposed to 3,3',4,4'-Tetrachlorobiphenyl (Tcb), *Bulletin of Environmental Contamination and Toxicology,* 56, 782, 1996.

216. **Freeman, H.C. and Idler, D.R.**, Effect of Polychlorinated Biphenyl on Steroidogenesis and Reproduction in Brook Trout (Salvelinus-Fontinalis), *Canadian Journal of Biochemistry,* 53, 666, 1975.

217. **Ankley, G., Tillitt, D., Giesy, J., Jones, P., and Verbrugge, D.**, Bioassay-Derived 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin Equivalents in PCB-Containing Extracts From the Flesh and Eggs of Lake Michigan Chinook Salmon (*Oncorhynchus Tshawytscha*) and Possible Implications for Reproduction., *Canadian Journal of Fisheries and Aquatic Sciences,* 48, 1685, 1991.

218. **Ungerer, J. and Thomas, P.**, Transport and Accumulation of Organochlorines in the Ovaries of Atlantic Croaker (*Micropogonias Undulatus*), *Marine Environmental Research,* 42, 167, 1996.

219. **Sangalang, G.B., Freeman, H.C., and Crowell, R.**, Testicular Abnormalities in Cod (Gadus-Morhua) Fed Aroclor-1254, *Archives of Environmental Contamination and Toxicology,* 10, 617, 1981.

220. **Suedel, B.C., Dillon, T.M., and Benson, W.H.**, Subchronic Effects of Five Di-Ortho Pcb Congeners on Survival, Growth and Reproduction in the Fathead Minnow Pimephales Promelas, *Environmental Toxicology and Chemistry,* 16, 1526, 1997.

221. **Thomas, P.**, Changes in the Plasma-Levels of Maturation-Inducing Steroids in Several Perciform Fishes During Induced Ovulation, *American Zoologist,* 28, A53-A53, 1988.

222. **Dicarlo, F.J., Seifter, J., and Decarlo, V.J.**, Assessment of Hazards of Polybrominated Biphenyls, *Environmental Health Perspectives,* 23, 351, 1978.

223. **Roboz, J., Greaves, J., and Bekesi, J.G.**, Polybrominated Biphenyls in Model and Environmentally Contaminated Human-Blood - Protein-Binding and Immunotoxicological Studies, *Environmental Health Perspectives,* 60, 107, 1985.

224. **Roboz, J., Greaves, J., Mccamish, M., Holland, J.F., and Bekesi, G.**, An Invitro Model for the Binding of Polybrominated Biphenyls in Environmentally Contaminated Blood, *Archives of Environmental Contamination and Toxicology,* 14, 137, 1985.

225. **Hesse, J.L. and Powers, R.A.**, Polybrominated Biphenyl (Pbb) Contamination of Pine River, Gratiot, and Midland Counties, Michigan, *Environmental Health Perspectives,* 23, 19, 1978.

226. **Damstra, T., Jurgelski, W., Posner, H.S., Vouk, V.B., Bernheim, N.J., Guthrie, J., Luster, M., and Falk, H.L.**, Toxicity of Polybrominated Biphenyls (Pbbs) in Domestic and Laboratory-Animals, *Environmental Health Perspectives*, 44, 175, 1982.

227. **Polin, D. and Ringer, R.K.**, Polybrominated Biphenyls in Chicken Eggs Vs Hatchability, *Proceedings of the Society for Experimental Biology and Medicine*, 159, 131, 1978.

228. **Polin, D. and Ringer, R.K.**, Pbb Fed to Adult Female Chickens - Its Effect on Egg- Production, Reproduction, Viability of Offspring, and Residues in Tissues and Eggs, *Environmental Health Perspectives*, 23, 283, 1978.

229. **Zitko, V.**, Accumulation of Polybrominated Biphenyls by Fish, *Bulletin of Environmental Contamination and Toxicology*, 17, 285, 1977.

230. **Kuehl, D., Butterworth BC, McBride A, Kroner, S., and Bahnick D.**, Contamination of Fish by 2,3,7,8-Tetrachlorodibenzo-p-Dioxin - A Survey of Fish From Major Watersheds in the United States, *Chemosphere*, 18, 1997, 1989.

231. **Birnbaum, L. and Tuomisto, J.**, Non-Carcinogenic Effects of TCDD in Animals, *Food Additives and Contaminants*, 17, 275, 2000.

232. **Boening, D.**, Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin to Several Ecological Receptor Groups: a Short Review, *Ecotoxicology and Environmental Safety*, 39, 155, 1998.

233. **Mably, T., Bjerke, D., Moore, R., Gendronfitzpatrick, A., and Peterson, R.**, In Utero and Lactational Exposure of Male Rats to 2,3,7,8-Tetrachlorodibenzo-p-Dioxin:3. Effects on Spermatogenesis and Reproductive Capacity, *Toxicology and Applied Pharmacology*, 114, 118, 1992c.

234. **Mably, T., Moore, R., Goy, R., and Peterson, R.**, In Utero and Lactational Exposure of Male Rats to 2,3,7,8-Tetrachlorodibenzo-p-Dioxin:2. Effects on Sexual Behavior and the Regulation of Luteinizing Hormone Secretion in Adulthood, *Toxicology and Applied Pharmacology*, 114, 108, 1992b.

235. **Mably, T., Moore, R., and Peterson, R.**, In Utero and Lactational Exposure of Male Rats to 2,3,7,8-Tetrachlorodibenzo-p-Dioxin:1. Effects on Androgenic Status, *Toxicology and Applied Pharmacology*, 114, 108, 1992a.

236. **Gray, L., Kelce, W., Monosson, E., Ostby, J., and Birnbaum, L.**, Exposure to TCDD During Development Permanently Alters Reproductive Function in Male Longevans Rats and Hamsters-Reduced Ejaculated and Epididymal Sperm Numbers and Sex Accesory-Gland Weights in Offspring With Normal Androgenic Status, *Toxicology and Applied Pharmacology*, 131, 108, 1995.

237. **Peterson, R.E., Theoblad, H.M., and Kimmel, G.L.**, Developmental and Reproductive Toxicity of Dioxins and Related Compounds: Cross-Species Comparisons., *Critical Reviews in Toxicology*, 23, 283, 1993.

238. **Hoffman, D., Rice, C., and Kubiak, T.**, PCBs and Dioxins in Birds, in *Environmental Contaminants in*

Wildlife. Interpreting Tissue Concentrations, Beyer, W., Heinz, G., and Redmon-Norwood, A., Eds., CRC Lewis Publishers, Boca Raton, FL, 1996, 165.

239. **Gilbertson, M., Kubiak, T., and Ludwig, J.**, Great Lakes Embryo Mortality, Edema and Deformities Syndrome (GLEMEDS) in Colonial Fish-Eating Birds: Similarity to Chick-Edema Disease, *Journal of Toxicology and Environmental Health,* 455, 1991.

240. **Giesey, J., Ludwig, J., and Tillitt, D.**, Deformities in Birds of the Great Lakes Region, *Environmental and Science Technology,* 28, 128, 1994.

241. **Grasman, K., Scanlon, P., and Fox, G.**, Reproductive and Physiological Effects of Environmental Contaminants in Fish-Eating Birds of the Great Lakes: a Review of Historical Trends, *Environmental Monitoring and Assessment,* 53, 117, 1998.

242. **Ludwig, J., KuritaMatsuba, H., Auman, H., Ludwig, M., Summer, C., Glesy, J., Tillitt, D., and Jones, P.** , Deformities, PCBs, and TCDD-Equivalents in Double-Crested Cormorants (Phalacrocorax Auritus) and Caspian Terns (Hydroprogne Caspia) of the Upper Great Lakes 1986-91: Testing a Cause-Effect Hypothesis, *Journal of Great Lakes Research,* 22, 172, 1996.

243. **Heinz, G.,** Contaminant Effects on Great Lakes Fish-Eating Birds: a Population Perspective, in *Principles and Processes for Evaluating Endocrine Disruption in Wildlife,* Kendall, R.J., Dickerson, R.L., Giesy, J.P., and Suk, W.P., Eds., SETAC Press, Pensacola, FL, 1998, 141.

244. **Hart, L., Cheng, K., Whitehead, P., Shah, R., Lewis, R., Ruschlowski, S., Blair, R., Bennett, D., Bandiera, S., Norstrom, R., and Bellward, G.**, Dioxin Contamination and Grwoth and Development in Great Blue Heron Embryos, *Journal of Toxicology and Environmental Health,* 32, 331, 1991.

245. **Elliott, J., Butler, R., Norstrom, R., and Whitehead, P.**, Environmental Contaminants and Reproductive Success of Great Blue Herons (Ardea Herodias) in British Columbia, 1986-1989, *Environmental Pollution,* 59, 91, 1989.

246. **Sanderson, J., Nosrtrom, R., Elliott, J., Hart, L., Cheng, K., and Bellward, G.**, Biological Effects of Polychlorinated Bidenzo-p-Dioxins, Dibenzofurans, and Biphenyls in Double-Crested Cormorant Chicks (Phalacrocorax Auritus), *Journal of Toxicology and Environmental Health,* 41, 247, 1994.

247. **White, D. and Seginak, J.**, Dioxins and Furans Linked to Reproductive Impairment in Wood Ducks at Bayou Meto, Arkansas, *Journal of Wildlife Management,* 58, 100, 1994b.

248. **Henshel, D.**, Developmental Neurotoxic Effects of Dioxin and Dioxin-Like Compounds on Domestic and Wild Avain Species, *Environmental Toxicology and Chemistry,* 17, 88, 1998.

249. **Powell, D., Aulerich, R., Meadows, J., Tillitt, D., Kelly, M., Stromborg, K., Melancon, M., Fitzgerald, S., and Bursian, S.**, Effects of 3,3', 4,4', 5-Pentachlorobiphenyl and 2,3,7,8-Tetrachlorodibenzo-p-

Dioxin Injected into the Yolks of Double-Crested Cormorant (Phalacrocorax Auritus) Eggs Prior to Incubation, *Environmental Toxicology and Chemistry,* 17, 2035, 1998.

250. **Rolland, R.**, A Review of Chemically-Induced Alterations in Thyroid and Vitamin A Status From Field Studies of Wildlife and Fish, *Journal of Wildlife Diseases,* 36, 615, 2000.

251. **Murk, A., Boudewijn, T., Meininger, P., Bosveld, A., Rossaert, G., and Ysebaert, P.**, Effects of Polyhalogenated Aromatic Hydrocarbons and Related Contaminants on Common Tern Reproduction: Integration of Biological, Biochemical, and Chemical Data, *Archives of Environmental Contamination and Toxicology,* 31, 128, 1996.

252. **Van der Berg, M., Craane, B., Sinnige, T., Van Mourik, S., Dirksen, S., Boudewijn, T., Van Der Gaag, M., Lutke-schipolt, I., Spenkelink, B., and Brouwer, A.**, Biochemical and Toxic Effects of Polychlorinated Biphenyls (PCB's). Dibenzo-p-Dioxins (PCDD's) and Dibenzofurans (PCDF's) in the Cormorant (Phalacrocorax Carbo) After in Ovo Exposure, *Environmental Toxicology and Chemistry,* 13, 803, 1994.

253. **Janz, D. and Bellward, G.**, Effects of 2,3,7,8-Tetrachloro-p-Dioxin Exposure on Plasma Thyroid and Sex Steroid Hormone Concentrations and Estrogen Receptor Levels in Adult Great Blue Herons, *Environmental Toxicology and Chemistry,* 16, 985, 1997.

254. **Janz, D. and Bellward, G.**, In Ovo 2,3,7,8-Tetrachlorodibenzo-p-Dioxin Exposure in Three Avian Species. 1. Effects on Thyroid Hormones and Growth During Perinatal Periodthe Perinatal Period, *Toxicology and Applied Pharmacology,* 139, 281, 1996a.

255. **Gould, J., Cooper, K., and Scanes, C.**, Effects of Polychlorinated Biphenyl Mixtures and Three Specific Congeneres on Growth and Circulating Growth-Related Hormones, *General and Comparative Endocrinology,* 106, 221, 1997.

256. **Jung, R. and Walker, M.** , Effects of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) on Development of Anuran Amphibians, *Environmental Toxicology and Chemistry,* 16, 230, 1997.

257. **Karchner, S., Kennedy, S., Trudeau, S., and Hahn, M.**, Towards Molecular Understanding of Species Differences in Dioxin Sensitivity: Initial Characterization of Ah Receptor CDNAs in Birds and an Amphibian, *Marine Environmental Research,* 50, 51, 2000.

258. **Bishop, C.A., Ng, P., Petit, K.E., Kennedy, S.W., Stegeman, J.J., Norstrom, R.J., and Brooks, R.J.**, Environmental Contamination and Developmental Abnormalities in Eggs and Hatchlings of the Common Snapping Turtle (Chelydra Serpentina Serpentina) From the Great Lakew St. Lawrence River Basin (1989-91), *Environmental Pollution,* 101, 143, 1998.

259. **Matter, J., McMurry, C., Anthony, A., and Dickerson, R.**, Development and Implementation of Endocrine Biomarkers of Exposure and Effects in American Alligators (Alligator Mississippiensis), *Chemosphere,* 37, 1905, 1998.

260. **Cooper, K.**, The Effects of Polychlorinated Dibenzo-p-Dioxins and Polychlorinated Dibenzofurans on Aquatic Organisms, *CRC Critical Reviews in Aquatic Sciences,* 1, 227, 1989.

261. **Walker, M.K. and Peterson, R.E.,** Aquatic Toxicity of Dioxins and Related Chemicals, in *Dioxins and Health,* Schecter, A., Eds., Plenum Press, New York, 1994, 347.

262. **Walker, M. and Peterson, R.**, Potencies of Polychlorinated Dibenzo-*p*-Dioxin, Dibenzofuran, and Biphenyl Congeners, Relative to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin, for Producing Early Life Stage Mortality in Rainbow Trout (*Oncorhynchus Mykiss*), *Aquatic Toxicology,* 21, 219, 1991.

263. **Walker, M.K., Spitsbergen, J.M., Olson, J.R., and Peterson, R.E.**, 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) Toxicity During Early Life Stage Development of Lake Trout (*Salvelinus Namaycush*), *Canadian Journal of Fisheries and Aquatic Sciences,* 48, 875, 1991.

264. **Wisk, J. and Cooper, K.** , The Stage Specific Toxicity of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin in Embryos of the Japanese Medaka (*Oryzias Latipes*), *Environmental Toxicology and Chemistry,* 9, 1159, 1990.

265. **Wannemacher, R., Rebstock, A., Kulzer, E., Schrenk, D., and Bock, K.W.**, Effects of 2,3,7,8-Tetrachlorodibenzo-Dioxin on Reproduction and Oogenesis in Zebrafish (*Brachydanio Rerio*), *Chemosphere,* 24, 1361, 1992.

266. **Walker, M.K. and Peterson, R.E.,** Aquatic Toxicity of Dioxins and Related Chemicals, in *Dioxins and Health,* Schecter, A., Eds., Plenum Press, New York, 1994, 347.

267. **Giesy, J.P. and Snyder, E.M.,** Xenobiotic Modulation of Endocrine Function in Fishes, in *Principles and Processes for Valuating Endocrine Disruption in Wildlife.*, Eds., SETAC Press, Pensacola, FL, 1998, 155.

268. **Henry, T.R., Spitsbergen, J.M., Hornung, M.W., Abnet, C.C., and Peterson, R.E.**, Early Life Stage Toxicity of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin in Zebrafish (*Danio Rerio*), *Toxicology and Applied Pharmacology,* 142, 56, 1997.

269. **Peterson, R., Theoblad, H., and Kimmel, G.**, Developmental and Reproductive Toxicity of Dioxins and Related Compounds: Cross-Species Comparisons., *Critical Reviews in Toxicology,* 23, 283, 1993.

270. **Safe, S.H.,** 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Environmental Antiestrogens: Characterization and Mechanism of Action, in *Endocrine Disruptors. Effects on Male and Female Reproductive Systems.*, Eds., CRC, Boca Raton, FL, 1999, 187.

271. **Smeets, J., van Holsteijn, I., Giesy, J., and van den Berg , M.**, The Anti-Estrogenicity of Ah Receptor Agonists in Carp (Cyprinus Carpio) Hepatocytes, *Toxicological Sciences,* 52, 178, 1999.

272. **Kharat, I. and Saatcioglu, F.**, Antiestrogenic Effects of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin Are Mediated

by Direct Transcriptional Interference With the Liganded Estrogen Receptor - Cross-Talk Between Aryl Hydrocarbon- and Estrogen-Mediated Signaling, *Journal of Biological Chemistrry,* 271, 10533, 1996.

273. **Sanderson, J., Janz, D., Bellward, G., and Giesy, J.**, Effects of Embryonic and Adult Exposure to 2,3,7,8-Tetrachloro-p-Dioxin on Hepatic Microsomal Testosterone Hydroxylase Activities in Great Blue Herons (Ardea Herodias), *Environmental Toxicology and Chemistry,* 16, 1304, 1997.

274. **Janz, D. and Bellward, G.**, In Ovo 2,3,7,8-Tetrachlorodibenzo-p-Dioxin Exposure in Three Avian Species. 2. Effects on Estrogen Receptor and Plasma Sex Setroid Hormones During the Perinatal Period, *Toxicology and Applied Pharmacology,* 139, 292, 1996b.

275. **Bookstaff, R., Moore, R., and Peterson, R.**, 2,3,7,8-Tetrachlorodibenzo-p -Dioxin in Male Rats: Different Effects of in Utero Versus Lactational Exposure, *Toxicology and Applied Pharmacology,* 104, 212, 1990.

276. **Spear, P., Bilodeau, A., and Branchaud, A.**, Retiboids: From Metabolism to Environmental Monitoring, *Chemosphere,* 25, 1733, 1992.

277. **Brouwer, A., Morse, D., Lans, M., Schuur, A., Murk, A., Klasson-wehler, E., Bergman, A., and Visser, T.**, Interactions of Persistent Environmental Organohalogens and the Thyroid Hormone System: Mechanisms and Possible Consequences for Animal and Human Health, in *Endocrine-Disrupting Chemicals: Neural, Endocrine, and Behavioral Effects,* Colborn, T., vom Saal, F., and Short, P., Eds., 1998, 59.

278. **Lans, M., Klassonwehler, E., Willemsen, M., Meussen, E., Safe, S., and Brouwer, A.**, Structure-Dependent, Competitive Interaction of Hydroxy-Polychlorinatedbiphenyls, Hydroxy-Dibenzo-p-Dioxins and Hydroxy-Dibenzofurans With Human Transthyretin, *Chemico-Biological Interactions,* 88, 7, 1993.

279. **Cantrell, S.M., Lutz, L.H., Tillitt, D.E., and Hannink, M.**, Embryotoxicity of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD): the Embryonic Vasculature Is a Physiological Target for TCDD-Induced DNA Damage and Apoptotic Cell Death in Medaka (*Orizias Latipes*), *Toxicology and Applied Pharmacology,* 141, 23, 1996.

280. **Goodbred, S. L., R. J. Gilliom, T. S. Gross, N. P. Denslow, W. L. Bryant, and T. R. Schoeb.** *Reconnaissance of 17ß-Estradiol, 11-Ketotestosterone, Vitellogenin, and Gonad Histopathology in Common Carp of United States Streams: Potential for Contaminant-Induced Endocrine Disruption*, USGS Open-File Report nr 96-627. U. S. Geological Survey, Sacramento (CA), 1997.

281. **Gross, D.A., Gross, T.S., Johnson, B., and Folmar, L.**, Characterization of Endocrine-Disruption and Clinical Manifestations in Largemouth Bass From Florida Lakes [Abstract], in *Abstract Book of the Society of Environmental Toxicology and Chemistry's Second SETAC World Congress (16th Annual Meeting); Global Environmental Protection: Science, Politics, and Common Sense.*, Eds., SETAC Press, Pensacola (FL), 1995, 185.

282. **Pickering, O.H., Henderson, C., and Lemke, A.E.**, The Toxicity of Organic Phosphorus Insecticides to Different Species of Warmwater Fishes, *Transactions of the American Fisheries Society,* 91, 175, 1962.

283. **Voldner, E.C. and Li, Y.F.**, Global Usage of Toxaphene, *Chemosphere,* 27, 2073, 1993.

284. **Glassmeyer, S.T., Devault, D.S., Myers, T.R., and Hites, R.A.**, Toxaphene in Great Lakes Fish: a Temporal, Spatial, and Trophic Study, *Environmental Science & Technology,* 31, 84, 1997.

285. **Delorme, P.D., Muir, D.C.G., Lockhart, W.L., Mills, K.H., and Ward, F.J.**, Depuration of Toxaphene in Lake Trout and White Suckers in a Natural Ecosystem Following a Single Ip Dose, *Chemosphere,* 27, 1965, 1993.

286. **Naqvi, S.M. and Vaishnavi, C.**, Bioaccumulative Potential and Toxicity of Endosulfan Insecticide to Non-Target Animals, *Comparative Biochemistry and Physiology,* 105C, 347, 1993.

287. **Fowler, J.F., Newsom, L.D., Graves, J.B., Bonner, F.L., and Schillin.pe.**, Effect of Dieldrin on Egg Hatchability, Chick Survival and Eggshell Thickness in Purple and Common Gallinules, *Bulletin of Environmental Contamination and Toxicology,* 6, 495-&, 1971.

288. **Lockie, J.D., Ratcliff.da, and Balharry, R.**, Breeding Success and Organo-Chlorine Residues in Golden Eagles in West Scotland, *Journal of Applied Ecology,* 6, 381-&, 1969.

289. **Fleming, W.J., McLane, M., and Cromartie, E.**, Endrin Decreases Screech Owl Productivity, *Journal of Wildlife Management,* 46, 462, 1982.

290. **Blus, L.J., Henny, C.J., Lenhart, D.J., and Kaiser, T.E.**, Effects of Heptachlor-and Lindane-Treated Seed on Canada Geese, *Journal of Wildlife Management,* 48, 1097, 1984.

291. **Henny, C.J., Blus, L.J., and Stafford, C.J.**, Effects of Heptachlor on American Kestrels in the Columbia Basin, Oregon, *Journal of Wildlife Management,* 47, 1080, 1983.

292. **Fyfe, R.W., Risebrough, R.W., and Walker, W.I.**, Pollutant Effects on the Reproduction of the Prairie Falcons and Merlins of the Canadian Prairies, *The Canadian Field-Naturalist,* 90, 346, 1976.

293. **Spann, J. W.** "Unpublilshed Data." *Other Organochlorine Pesiticides in Birds* Stanley N. Wiemeyereds.W. Nelson Beyer, Gary H. Heinz, and Amy W. Redmon-Norwood, Boca Raton, Fl: Lewis Publishers, 1996.

294. **Bush, P.B., Kiker, J.T., Page, R.K., Booth, N.H., and Fletcher, O.J.**, Effects of Graded Levels of Toxaphene on Poultry Residue Accumulation, Egg-Production, Shell Quality, and Hatchability in White Leghorns, *Journal of Agricultural and Food Chemistry,* 25, 928, 1977.

295. **Heinz, G.H. and Finley, M.T.**, Toxaphene Does Not Affect Avoidance-Behavior of Young Black Ducks, *Journal of Wildlife Management,* 42, 408, 1978.

296. **Willingham, E. and Crews, D.**, Sex Reversal Effects of Environmentally Relevant Xenobiotic Concentrations on the Red-Eared Slider Turtle, a Species With Temperature-Dependent Sex Determination, *General and Comparative Endocrinology,* 113, 429, 1999.

297. **Faahraeus-Van Ree, G.E. and Payne, J.F.**, Effect of Toxaphene on Reproduction of Fish, *Chemosphere,* 34, 855, 1997.

298. **Smith, R.M. and Cole, C.F.**, Effects of Egg Concentrations of Ddt and Dieldrin on Development in Winter Flounder (Pseudopleuronectes-Americanus), *Journal of the Fisheries Research Board of Canada,* 30, 1894, 1973.

299. **Hermanutz, R.O.**, Endrin and Malathion Toxicity to Flagfish (Jordanella Floridae), *Archives of Environmental Contamination and Toxicology,* 7, 159, 1978.

300. **Matthiessen, P. and Logan, J.W.M.**, Low Concentration Effects of Endosulfan Insecticide on Reproductive-Behavior in the Tropical Cichlid Fish Sarotherodon-Mossambicus, *Bulletin of Environmental Contamination and Toxicology,* 33, 575, 1984.

301. **Arora, N. and Kulshrestha, S.K.**, Comparison of the Toxic Effects of 2 Pesticides on the Testes of a Fresh-Water Teleost Channa-Striatus Bl, *Acta Hydrochimica Et Hydrobiologica,* 12, 435, 1984.

302. **Kumar, S. and Pant, S.C.**, Comparative Sublethal Ovarian Pathology of Some Pesticides in the Teleost, Puntius-Conchonius Hamilton, *Bulletin of Environmental Contamination and Toxicology,* 41, 227, 1988.

303. **Kulshrestha, S.K. and Arora, N.**, Impairments Induced by Sublethal Doses of 2 Pesticides in the Ovaries of a Fresh-Water Teleost Channa-Striatus Bloch, *Toxicology Letters,* 20, 93, 1984.

304. **Rastogi, A. and Kulshrestha, S.K.**, Effect of Sublethal Doses of 3 Pesticides on the Ovary of a Carp Minnow Rasbora-Daniconius, *Bulletin of Environmental Contamination and Toxicology,* 45, 742, 1990.

305. **Chakravorty, S., Lal, B., and Singh, T.P.**, Effect of Endosulfan (Thiodan) on Vitellogenesis and Its Modulation by Different Hormones in the Vitellogenic Catfish *Clarias Batrachus*, *Toxicology,* 75, 191, 1992.

306. **Inbaraj, R.M. and Haider, S.**, Effect of Malathion and Endosulfan on Brain Acetylcholinesterase and Ovarian Steroidogenesis of Channa- Punctatus (Bloch), *Ecotoxicology and Environmental Safety,* 16, 123, 1988.

307. **Shukla, L. and Pandey, A.K.**, Effects of Endosulfan on the Hypothalamo Hypophyseal Complex and Fish Reproductive Physiology, *Bulletin of Environmental Contamination and Toxicology,* 36, 122, 1986.

308. Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for DDT, DDE, and DDD, Draft Report*, U. S. Department of Health and Human Services, Atlanta, 1992.

309. ———. *Toxicological Profile for Mirex and Chlordecone*, U. S. Department of Health and Human Services, Atlanta, 1995.

310. **Guzelian, P.S.**, Comparative Toxicology of Chlordecone (Kepone) in Humans and Experimental-Animals, *Annual Review of Pharmacology and Toxicology,* 22, 89, 1982.

311. **Yamamoto, J.T., Donohoe, R.M., Fry, D.M., Golub, M.S., and Donald, J.M.,** Environmental Estrogens:Implications for Reproduction in Wildlife, in *Noninfectious Diseases of Wildlife,* Fairbrother A., Locke, L.N., and Hoff, G.L., Eds., Iowa state University Press, Ames, IA, 1996, 31.

312. **Huber, J.**, Some Physiologic Effects of the Insecticide Kepone in the Laboratory Mouse, *Toxicology and Applied Pharmacology*, 7, 516, 1965.

313. **Hammond, B., Katzenellenbogen, B.S., Krauthammer, N., and McConnel, J.**, Estrogenic Activity of the Insecticide Chlordecone (Kepone) and Interaction With Uterine Estrogen Receptors, *Proceedings National Academy of Science* , 76, 6641, 1979.

314. **Gellert, R.J.**, Uterotrophic Activity of Polychlorinated Biphenyls (Pcb) and Induction of Precocious Reproductive Aging in Neonatally Treated Female Rats, *Environmental Research*, 16, 123, 1978.

315. **McFarland, L.Z. and Lacy, P.B.**, Physiologic and Endocrinologic Effcts of the Insecticide Kepone in the Japanese Quail, *Toxicology and Applied Pharmacology*, 15, 441, 1969.

316. **Eroschenko, V.P.**, Alterations in Testes of Japanese Quail During and After Ingestion of Insecticide Kepone, *Toxicology and Applied Pharmacology*, 43, 535, 1978.

317. **Eroschenko, V.P.**, Morphological Alterations in Testes of Japanese Quail During and After Ingestion of Estrogenic Insecticide Kepone, *Anatomical Record*, 190, 391, 1978.

318. **Romanoff, A.L. and Romanoff, A.J.**, *The Avian Egg* , John Wiley & Sons, New York, 1949, <25 Page(s)>.

319. **Naber, E.C. and Ware, G.W.**, Effect of Kepone and Mirex on Reprodlctive Performance in Laying Hen, *Poultry Science*, 44, 875-&, 1965.

320. **Thomas, P. and Khan, I.A.**, Mechanisms of Chemical Interference With Reproductive Endocrine Function in Sciaenid Fishes, in *Chemically Induced Alterations in Functional Development and Reproduction of Fishes.*, Eds., SETAC Press, 1997, 29.

321. **Donohoe, R.M. and Curtis, L.R.**, Estrogenic Activity of Chlordecone, O,p'-DDT and O,p'-DDE in Juvenile Rainbow Trout: Induction of Vitellogenesis and Interaction With Hepatic Estrogen Binding Sites, *Aquatic Toxicology*, 36, 31, 1996.

322. **Thomas, P., Gosh, S., and Smith, J.S.**, Interactions of Organochlorines With Steroid Hormone Receptors in Atlantic Croaker, *Marine Environmental Research*, 42, 176, 1996.

323. **Nimrod, A.C. and Benson, W.H.**, Xenobiotic Interaction With and Alteration of Channel Catfish Estrogen Receptor, *Toxicology and Applied Pharmacology*, 147, 381, 1997.

324. **Curtis, L.R. and Beyers, R.J.**, Inhibition of Oviposition in Teleost Oryzias-Latipes, Induced by Sub-Acute Kepone Exposure, *Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology*, 61, 15, 1978.

325. **Srivastava, A.K. and Srivastava, A.K.**, Effects of Chlordecone on the Gonads of Freshwater Catfish, *Heteropneustes Fossilis*, *Bulletin of Environmental Contamination and Toxicology*, 53, 186, 1994.

326. **Bitman, J., Cecil, C., Jarros, S.J., and Fries, G.F.**, Estrogenic Activity of O,p'-DDT in the Mammalian Uterus and Avian Oviduct, *Science*, 162, 371, 1969.

327. **Clement, J.G. and Okey, A.B.**, Estrogenic and Antiestrogenic Effects of DDT Administered in the Diet to Immature Female Rats, *Canadian Journal of Physiology and Pharmacology,* 50, 971, 1972.

328. **Aguilar, A. and Borrell, A.**, Reproductive Transfer and Variation of Body Load of Organochlorine Pollutants With Age in Fin Whales *(Balaenoptera Physalus), Archives of Environmental Contamination and Toxicology,* 27, 548, 1994.

329. **Bowerman, W.W., Best, D.A., Grubb, T.G., Sikarskie, J.G., and Giesy, J.P.**, Assessment of Environmental Endocrine Disruptors in Bald Eagles of the Great Lakes, *Chemosphere,* 41, 1569, 2000.

330. **Lundholm, C.E.**, Dde-Induced Eggshell Thinning in Birds: Effects of P,P'-Dde on the Calcium and Prostaglandin Metabolism of the Eggshell Gland, *Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology,* 118, 113, 1997.

331. **Bishop, C.A., Collins, B., Mineau, P., Burgess, N.M., Read, W.F., and Risley, C.**, Reproduction of Cavity-Nesting Birds in Pesticide-Sprayed Apple Orchards in Southern Ontario, Canada, 1988-1994, *Environmental Toxicology and Chemistry,* 19, 588, 2000.

332. **Heinz, G.H., Percival, H.F., and Jennings, M.L.**, Contaminants in American Alligator Eggs From Lake Apopka, Lake Griffin, and Lake Okeechobee, Florida , *Environmental Monitoring and Assessment,* 16, 277, 1991.

333. **Woodward, A.R., Jennings, M.L., and Percival, H.F.**, Egg Collecting and Hatch Rates of American Alligator Eggs in Florida, *Wildlife Society Bulletin,* 17, 124, 1989.

334. **Woodward, A.R., Jennings, M.L., Percival, H.F., and Moore, J.F.**, Low Clutch Viability of American Alligators on Lake Apopka, *Florida Science,* 56, 52, 1993.

335. **Gross, T.S., Guillette, L.J., Percival, H.F., Masson, G.R., Matter, J.M., and Woodward, A.R.**, Contaminant Induced Reproductive Anomalies in Florida Alligators, *Comparitive Pathology Bulletin,* 4, 2, 1995.

336. **Palmer, B.D. and Palmer, S.K.**, Vitellogenin Induction by Xenobiotic Estrogens in the Red-Eared Turtle and the African Clawed Frog, *Environmental Health Perspectives,* 103, 19, 1995.

337. **Clark, E.J., Norris, D.O., and Jones, R.E.**, Interactions of Gonadal Steroids and Pesticides (DDT, DDE) on Gonaduct Growth in Larval Tiger Salamanders, Ambystoma Tigrinum, *General and Comparative Endocrinology,* 109, 94, 1998.

338. **Macek, K.J.**, Reproduction in Brook Trout (Salvelinus Fortinalis) Fed Sublethal Concentrations of Ddt, *Journal of the Fisheries Research Board of Canada,* 25, 1787-&, 1968.

339. **Burdick, G.E., Harris, E.J., Dean, H.J., Walker, T.M., Skea, J., and Colby, D.**, The Accumulation of DDT in Lake Trout and the Effect on Reproduction, *Transactions of the American Fisheries Society,* 93, 127, 1964.

340. **Hose, J.E., Cross, J.N., Smith, S.G., and Diehl, D.**, Reproductive Impairment in a Fish Inhabiting a Contaminated Coastal Environment Off Southern-California, *Environmental Pollution,* 57, 139, 1989.

341. **Smith, R.M. and Cole, C.F.**, Effects of Egg Concentrations of Ddt and Dieldrin on Development in Winter Flounder (Pseudopleuronectes-Americanus), *Journal of the Fisheries Research Board of Canada,* 30, 1894, 1973.

342. **Cross, J.N. and Hose, J.E.**, Evidence for Impaired Reproduction in White Croaker (*Genyonemus Lineatus*) From Contaminated Areas Off Southern California, *Marine Environmental Research,* 24, 185, 1988.

343. **Monod, G.**, Egg Mortality of Lake Geneva Charr (Salvelinus-Alpinus L) Contaminated by Pcb and Ddt Derivatives, *Bulletin of Environmental Contamination and Toxicology,* 35, 531, 1985.

344. **Spies, R.B., Thomas, P., and Matsui, M.**, Effects of DDT and PCB on Reproductive Endocrinology of *Paralabrax Clathratus* in Southern California, *Marine Environmental Research,* 42, 75, 1996.

345. Agency for Toxic Substances and Disease Registry (ATSDR). *Alpha-, Beta-, Gamma- and Delta-Hexachlorocyclohexane (Update)*, U. S. Department of Health and Human Services, Atlanta, 1994.

346. **Wester, P.W.**, Histopathological Effects of Environmental Pollutants □HCH and Methyl Mercury on Reproductive Organs in Freshwater Fish, *Comparative Biochemistry and Physiology,* 100C, 237, 1991.

347. **Wester, P.W., Canton, J.H., and Bisschoop, A.**, Histopathological Study of *Poecilia Reticulata* (Guppy) After Long-Term □-Hexachlorocyclohexane Exposure, *Aquatic Toxicology,* 6: 271, 1985.

348. **Wester, P.W. and Canton, J.H.**, Histopathological Study of *Oryzias Latipes* (Medaka) After Long-Term ß-Hexachlorocyclohexane Exposure, *Aquatic Toxicology,* 9, 21, 1986.

349. **Wester, P.W., Canton, J.H., and Bisschoop, A.**, Histopathological Study of *Poecilia Reticulata* (Guppy) After Long-Term □-Hexachlorocyclohexane Exposure, *Aquatic Toxicology,* 6: 271, 1985.

350. **Singh, P.B. and Singh, T.P.**, Impact of Gamma-BHC on Lipid Class Levels and Their Modulation by Reproductive Hormones in the Freshwater Catfish, *Heteropneustes Fossilis*, *Bulletin of Environmental Contamination and Toxicology,* 48, 23, 1992.

351. **Singh, P., Kime, D., Epler, P., and Chyb, J.**, Impact of -Hexachlorocyclohexane Exposure on Plasma Gonadotropin Levels and in Vitro Stimulation of Gonadal Steroid Production by Carp Hypophyseal Homogenate in *Carassius Auratus*, *Journal of Fish Biology,* 44, 195, 1994.

352. **Singh, S. and Singh, T.P.**, Impact of Malathion and Hexachlorocyclohexane on Plasma Profiles of Three Sex Hormones During Different Phases of the Reproductive Cycle in *Clarias Batrachus*, *Pesticide Biochemistry and Physiology,* 27, 301, 1987.

353. **Singh, P.B. and Singh, T.P.**, Impact of Gamma-BHC on Lipid Class Levels and Their Modulation by Reproductive Hormones in the Freshwater Catfish, *Heteropneustes Fossilis*, *Bulletin of Environmental Contamination and Toxicology,* 48, 23, 1992.

354. **Gray, L.E., Ostby, J., and Kelce, W.R.**, Developmental Effects of an Environmental Antiandrogen: the Fungicide Vinclozolin Alters Sex Differentiation of the Male Rat, *Toxicology and Applied Pharmacology,* 129, 46, 1994.

355. **Gray, L.Jr.**, Xenoendocrine Disrupters: Laboratory Studies on Male Reproductive Effects, *Toxicology Letters,* 102-103, 331, 1998.

356. **Hellwig, J.** "Reproduction Study With Reg No. 83-258 (Vinclozolin) in Rats Continuous Dietary Administration Over 2 Generations (2 Litters in the First and 2 Litters in the Second Generation)." Report #92/11251. Department of Toxicology, BASF AG, Ludwigshafen, 1992.

357. **Knuppen, R.** *Final Report: Study of the Binding of 3-(3,5-Dichlorophenyl)-5-Methylvinyl-2,4-Dion to the Androgen Receptor in MCF-7 Cells*, Institute of Biochemical Endocrinology, Medical University of Lubeck, 1990.

358. **Sheffield, SR.** "Effects of Field Exposure to Diazinon on Small Mammals Inhabiting a Semi-Enclosed Prairie Grassland Ecosystem. I. Ecological and Reproductive Effects. II. Sublethal Effects. " PhD Dissertation, Oklahoma State University., 1996.

359. **Pomeroy, S. and Barrett, G.**, Dynamics of Enclosed Small Mammal Populations in Relation to an Experimental Pesticide Application, *American Midland Naturalist,* 93, 91, 1975.

360. **Block, E.K., Lacher, T.E., Brewer, L.W., Cobb, G.P., and Kendall, R.J.**, Population Responses of Peromyscus Resident in Iowa Cornfields Treated With the Organophosphorus Pesticide Counter (R), *Ecotoxicology,* 8, 189, 1999.

361. **Rattner, B.**, Diagnosis of Anticholinesterase Poisoning in Birds: Effects of Environmental Temperature and Underfeeding on Cholinesterase Activity, *Environmental Toxicology and Chemistry,* 1, 329, 1982.

362. **Bennett, R.S., Williams B.A., Schmedding D.W., and Bennett J.K.**, Effects of Dietary Exposure to Methyl Parathion on Egg Laying and Incubation in Mallards, *Environmental Toxicology and Chemistry,* 10, 501, 1991.

363. **Vonier, P., Crain, D., McLachlan, J., Guillette, L., and Arnold, S.**, Interaction of Environmental Chemicals With the Estrogen and Progesterone Receptors From the Oviduct of the American Alligator, *Environmental Health Perspectives,* 104, 1318, 1996.

364. **White, D., Mitchell, C., and Hill, E.**, Parathion Alters Incubation Behavior of Laughing Gulls, *Bulletin of Enviornmentalk Contamination and Toxicology,* 31, 93, 1983.

365. **Stromborg, K.**, Seed Treatment of Pesticide Effects on Pheasant Reproduction at Sublethal Doses, *Journal of Wildlife Management,* 41, 632, 1977.

366. **Maitra, S.K. and Sarkar, R.**, Influence of Methyl Parathion on Gametogenic and Acetylcholinesterase Activity in the Testis of Whitethroated Munia (Lonchura Malabarica), *Archives of Environmental Contamination and Toxicology,* 30, 384, 1996.

367. **Hoffman, D.J.**, Embryotoxicity and Teratogenicity of Environmental Contaminants to Bird Eggs, *Reviews of Environmental Contamination and Toxicology,* 115, 39, 1990.

368. **Knapton, R. and Mineau, P.**, Effects of Granular Formulations of Terbufos and Fonofos Applied to Cornfields on Mortality and Reproductive Success of Songbirds, *Ecotoxicology,* 4, 138, 1995.

369. **Pascual, J., Peris, S., and Robredo, F.**, Efectos De Tratamientos Forestales Con Cipermetrina y Malation Sobre El Exito De Cria Del Herrerillo Comun (Parus Caeruleus), *Ecologia,* 359, 1991.

370. **Robinson, S., Kendall, R., Robinson, R., Driver, C., and Lacher, T.**, Effects of Agricultural Spraying of Methyl Parathion on Cholinesterase Activity and Reproductive Success in Wild Stralings (Sturnus Vulgaris), *Environmental Toxicology and Chemistry,* 7, 343, 1988.

371. **Powell, G.**, Reproduction by an Altricial Songbird, the Red-Winged Blackbird, in Fields Treated With the Organophosphate Insecticide Fenthion, *Journal of Applied Ecology,* 21, 83, 1984.

372. **Fordham, C., Tessari, J., Ramsdell, H., and Keefe, T.**, Effects of Malathion on Survival, Growth, Development, and Equilibrium Posture of Bullfrog Tadpoles (Rana Catesbeiana), *Environmental Toxicology and Chmeistry,* 20, 179, 2001.

373. **Harris, M., Bishop, C., Struger, J., Ripley, B., and Bogart, J.**, The Functional Integrity of Northern Leopard Frog (Rana Pipiens) and Green Frog (Rana Clamitans) Populations in Orchard Wetlands. II. Effects of Pesticides and Eutrophic Conditions on Early Life Stage Development, *Environmental Toxicology and Development,* 17, 1351, 1998.

374. **Alvarez, R., Honrubia, M., and Herraez, M.**, Skeletal Malformations Induced by the Insecticides ZZ-Aphox and Folidol During Larval Development of Rana Perezi, *Archives of Environmental Contamination and Toxicology,* 28, 349, 1995.

375. **Pauli, B., Coulson, D., and Berrill, M.**, Sensitivity of Amphibian Embryos and Tadpoles to Mimic(R) 240 LV Insecticide Following Single or Double Exposures, *Environmental Toxicology and Chemistry,* 18, 2538, 1999.

376. **Ram, R.N. and Sathyanesan, A.G.**, Ammonium Sulfate Induced Nuclear Changes in the Oocyte of the Fish, *Channa Punctatus* (BI)., *Bulletin of Environmental Contamination and Toxicology,* 36, 871, 1986.

377. **Choudhury, C., Ray, A., Bhattacharya, S., and Bhattacharya, S.**, Non-Lethal Concentrations of Pesticide Impair Ovarian Function in the Freshwater Perch, Anabas Testudineus, *Environmental Biology of Fishes,* 36, 319, 1993.

378. **Kumar, S. and Pant, S.**, Comparative Sublethal Ovarian Pathology of Some Pesticides in the Teleost,

Puntius Conchonius Hamilton, *Bulletin of Environmental Contamination and Toxicology,* 41, 227, 1988.

379. **Yasuno, M., Hatakeyama, S., and Miyashita, M.**, Reproduction in the Guppy (Poecilia Reticulata) Under Chronic Exposure to Mephos and Fenitrothion, *Bulletin of Environmental Contamination and Toxicology,* 25, 29, 1980.

380. **Haider, S. and Upadhyaya, N.**, Effect of Commercial Formulation of Four Organophosphorusinsecticides on the LH-Induced Germinal Vesicle Breakdown in the Oocytes of a Freshwater Teleost, Mystus Vittatus --A Preliminary in Vitro Study, *Ecotoxicology and Environmental Safety,* 161, 1986.

381. **Saxena, P. and Mani, K.** , Quantitative Study of Testicular Recrudescence in the Freshwater Teleost, Channa Punctatus Exposed to Pesticides. *Bulletin of Environmental Contamination and Toxicology,* 34, 597, 1985.

382. **Saxena, P. and Mani, K.** , Effect of Safe Concentrations of Some Pesticides on Testicular Recrudescence in the Freshwater Murrel, Channa Punctatus: a Morphological Study , *Ecotoxicoly and Environmental Safety,* 14, 56, 1987.

383. **Pandey, A. and Shukla, L.**, Effect of an Organophosphorous Insecticide Malathion on the Testicular Histophysiology in Sorathoradon Mossambicus , *National Academy of Science Letter,* 5, 141, 1982.

384. **Kling, D.**, Total Atresia of the Ovaries of Tilapia Leucosticta (Cichlidae) After Intoxication With the Insecticide Lebaycid , *Experientia,* 37, 73, 1981.

385. **Kapur, K., Kamaldeep, K., and Toor, H.**, The Effect of Fenitrothion on Reproduction of a Teleost Fish, Cyprinus Carpio Communis (Linn.): a Biochemical Study , *Bulletin of Environmental Contamination and Toxicology.,* 20, 438, 1978.

386. **Shukla, L., Shrivastava, A., Merwani, D., and Pandey, A.**, Effect of Sublethal Malathion on Ovarian Histophysiology in Sarotherodon Mossambicus , *Comparative Physiology and Ecology* , 9, 13, 1984.

387. **Singh, H.**, Effects of Malathion on Steroidogenesis and Sex Reversal in Monopterus Albus, *Marine Environmental Research,* 35, 159, 1993.

388. **Singh, H.**, Interaction of Xenobiotics With Reproductive Endocrine Functions in a Protogynous Teleost, Monopterus Albus, *Marine Environmental Research,* 28, 285, 1989.

389. **Pawar, K. and Katdare, M.**, Effects of Sublethal and Lethal Concentrations of Fenitrothion, BHC and Carbofuran on Behaviour and Oxygen Consumption of the Freshwater Prawn Macrobrachium Kistnensis (Tiwari) , *Archiv Fur Hydrobiologie,* 99, 398, 1984.

390. **Mani, K. and Saxena, P.** , Effect of Safe Concentrations of Some Pesticides on Ovarian Recrudescence in the Frehwater Murrel, Channa Punctatus (Bi).: a Quantitative Study , *Ecotoxicology and Environmental Safety,* 9, 241, 1985.

391. **Saxena PK and Garg, M.**, Effect of Insecticidal Pollution on Ovarian Recrudescence in the Fresh Water Teleost Channa Punctatus , *Indian Journal of Experimental Biology,* 16, 689, 1978.

392. **Lien, N., Adriaens, D., and Janssen, C.**, Morphological Abnormalities in African Catfish (Clarias Gariepinus) Larvae Exposed to Malathion, *Chemosphere,* 35, 1475, 1997.

393. **Dutta, H., Nath, A., Adhikari, S., Roy, P., Singh, N., and Datta Munshi, J.**, Sublethal Malathion Induced Changes in the Ovary of an Air-Breathing Fish, Heteropneustes Fossilis: a Histological Study, *Hydrobiologia,* 215, 1994.

394. **Singh, S. and Singh, T.** , Impact of Malathion and Hexachlorocyclohexane on Plasma Profiles of Three Sex Hormones During Different Phases of the Reproductive Cycle in Clarias Batrachus , *Pesticide and Biochemical Physiology,* 27, 301, 1987.

395. **Sinha, N., Lal, B., and Singh, T.**, Carbaryl-Induced Thyroid Dysfunction in the Freshwater Catfish Clarias Batrachus, *Ecotoxicology and Environmental Safety,* 213, 240, 1991.

396. **Ghosh, P., Bhattacharya, S., and Bhattacharya, S.**, Impact of Nonlethal Levels of Metacid-50 and Carbaryl on Thyroid Function and Cholinergic System of Channa Punctatus, .*Biomedical and Environmental Sciences* , 2, 92, 1989.

397. **Moore, A. and Waring, C.**, Sublethal Effects of the Pesticide Diazinon on Olfactory Function in Mature Male Atlantic Salmon Parr, *Journal of Fish Biology,* 48, 758, 1996.

398. **Singh, H. and Singh, T.** , Thyroid Activity and TSH Potency of the Pituitary Galnd and Blood Serum in Response to Cythion and Hexadrin Treatment in the Freshwater Catfish, Heteropneustes Fossilis (Bloch) , *Environmental Research,* 22, 184, 1980.

399. **Bagchi, P., Chatterjee, S., Ray, A., and Deb, C.**, Effect of Quinalphos, Organophosphorous Insecticide, on Testicular Steroidogenesis in Fish, Clarias Batrachus , *Bulletin of Environmental Contamination and Toxicology,* 44, 871, 1990.

400. **Singh, P.**, Impact of Malathion and Â-BHC on Lipid Metabolism in the Freshwater Female Catfish, Heteropneustes Fossilis , *Ecotoxicology and Environmental Safety,* 23, 22, 1992.

401. **Lal, B. and Singh, T.**, Impact of Pesticides on Lipid Metabolism in the Freshwater Catfish, Clarias Batrachus, During the Vitellogenic Phase of Its Annual Reproductive Cycle., *Ecotoxicology and Environmental Safety,* 13, 13, 1987.

402. **Bridges, D.W., Cech J.J.Jr., and Pedro D.N.**, Seasonal Hematological Changes in Winter Flounder, Pseudopleuronectes Americanus, *Transactions of the American Fisheries Society,* 5, 596, 1976.

403. **Chatterjee, S., Dutta, A., and Ghosh, R.**, Impact of Carbofuran in the Oocyte Maturation of Catfish, Heteropenustes Fossilis (Bloch) , *Archives of Environmental Contamination and Toxicology,* 32, 426, 1997.

404. **Rastogi, A. and Kulshrestha, S.**, Effect of Sublethal Doses of Three Pesticides on the Ovary of a Carp Minnow, Rasbora Daniconius , *Bulletin of Environmental Contamination and Toxicology.,* 45, 742, 1990.

405. **Sukumar, A. and Karpagaganapathy, P.**, Pesticide-Induced Atresia in Ovary of a Fresh Water Fish, Colisa Lalia , *Bulletin of Environmental Contamination and Toxicology* , 48, 357, 1992.

406. **Saxena PK and Garg, M.**, Effect of Insecticidal Pollution on Ovarian Recrudescence in the Fresh Water Teleost Channa Punctatus , *Indian Journal of Experimental Biology,* 16, 689, 1978.

407. **Kulshrestha, S. and Arora, N.**, Impairments Induced by Sublethal Doses of Two Pesticides in the Ovaries of a Freshwater Teleost Channa Striatus, *Toxicological Letters,* 20, 93, 1984.

408. **Carlson, A.**, Effects of Long-Term Exposure to Carbaryl (Sevin) on Survival, Growth, and Reproduction of the Fathead Minnow (Pimephales Promelas) , *Journal of Fisheries and Research Board of Canada,* 29, 583, 1972.

409. **Sonnenschein, C. and Soto, A.**, An Updated Review of Environmental Estrogen and Androgen Mimics and Antagonists, *Journal of Steroid Biochemistry,* 65, 143, 1998.

410. **Saxena, P., Mani, K., and Kondal, J.**, Effect of a Safe Application Rate (SAR) Concentrations of Some Biocides on the Gonads of the Fresh Water Murrel, Channa Punctatus--A Biochemical Study , *Ecotoxicology and Environmental Safety* , 12, 1, 1986.

411. **Matthiessen, P. and Gibbs, P.E.**, Critical Appraisal of the Evidence for Tributyltin-Mediated Endocrine Disruption in Mollusks, *Environmental Toxicology and Chemistry,* 17, 37, 1998.

412. **Gibbs, P., Pascoe, P., and Burt, G.**, Sex Change in the Female Dog-Whelk, Nucella Lapillus, Induced by Tributyltin From Antifouling Paints, *Journal of the MArine Biological Association of the United Kingdom,* 68, 715, 1988.

413. **Spooner, N., Gibbs, P., Bryan, G., and Goad, L.**, The Effect of Tributiltin Upon Steroid Titres in the Female Dogwhelk, Nucella Lapillus, and the Development of Imposex, *Marine Environmental Research,* 32, 37, 1991.

414. **Bryan, G., Gibbs, P., Burt, G., and Hummerstone, L.**, The Effects of Tributyltin (TBT) Acumulation on Adult Dog-Whelks, Nucella Lapillus: Long-Term Field and Laboratory Experiments, *Journal of the*

Marine Biological Association of the United Kingdom, 67, 525, 1987.

415. **Gibbs, P., Spencer, B., and Pascoe, P.**, The American Oyster Drill, Urosalpinx Cinerea (Gastropoda): Evidence of Decline in an Imposex-Affected Population (R. Blackwater, Essex) , *Journal of the Marine Association of the United Kingdom,* 71, 827, 1991.

416. **Ruiz, J., Bryan, G., and Gibbs, P.**, Effects of Tributyltin (TBT) Exposure on the Veliger Larvae Development of the Bivalve Scrobicularia Plana (Da Costa), *Journal of Experimental Marine Biology and Ecology,* 186, 53, 1995.

417. **Depledge, M.H. and Billinghurst, Z.**, Ecological Significance of Endocrine Disruption in Marine Invertebrates, *Marine Pollution Bulletin,* 39, 32, 1999.

418. **Martin, R., Dixon, D., Maguire, R., Hodson, P., and Tkacz, R.**, Acute Toxicity, Uptake, Depuration and Tissue Distribution of Tri-n-Butyltin in Rainbow Trout, Salmo Gairdneri, *Aquatic Toxicology,* 15, 37, 1989.

419. **Strmac, M. and Braunbeck, T.**, Effects of Triphenyltin Acetate on Survival, Hatching Success, and Liver Ultrastructure of Early Life Stages of Zebrafish (Danio Rerio), *Ecotoxicology and Environmental Safety* , 44, 25, 1999.

420. **Nirmala, K., Oshima, Y., Lee, R., Imada, N., Honjo, T., and Kobayashi, K.**, Transgenerational Toxicity of Tributyltin and Its Combined Effects With Polychlorinated Biphenyls on Reproductive Processes in Japanese Medaka (Oryzias Latipes), *Environmental Toxicology and Chemistry,* 18, 717, 1999.

421. **Manning, C., Lytle, T., Walker, W., and Lytle, J.**, Life-Cycle Toxicity of Bis(Tributyltin) Oxide to the Sheepshead Minnow (Cyprinodon Variegatus), *Archives of Environmental Contamination and Toxicology* , 37, 258, 1999.

422. **Seinen, W., Helder, T., Vernij, H., Penninks, A., and Leeuwangh, P.**, Short Term Toxicity of Tri-n-Butyltin Chloride in Rainbow Trout (Salmo Gairdneri Richradson) Yolk Sac Fry, *Science of the Total Environment,* 19, 155, 1981.

423. **Pinkney, A., Matteson, L., and Wright, D.**, Effects of Tributyltin on Survival, Growth, Morphometry, and RNA-DNA Ratio of Larval Striped Bass, Morene Saxatilis, *Archives of Environmental Contamination and Toxicology,* 1990.

424. **Fent, K. and Meier, W.**, Tributyltin-Induced Effects on Early Life Stages of Minnows Phoxinus Phoxinus, *Archives of Environmental Toxicology and Chemistry,* 428, 1992.

425. **Haubruge, E., Petit, F., and Gage, M.J.G.**, Reduced Sperm Counts in Guppies (Poecilia Reticulata) Following Exposure to Low Levels of Tributyltin and Bisphenol a, *Proceedings of the Royal Society of London Series B-Biological Sciences,* 267, 2333, 2000.

426. **Holm, G., Norrgren, L., and Linden, O.**, Reproductive and Histopathological Effects of Long-Term Experimental Exposure to Bis(Tributyltin)Oxide (TBTO) on the Three-Spined Stickleback,

Gasterosteus Aculeatus Linnaeus., *Journal of Fish Biology,* 38, 373, 1991.

427. **Jha, A.N., Hagger, J.A., Hill, S.J., and Depledge, M.H.**, Genotoxic, Cytotoxic and Developmental Effects of Tributyltin Oxide (TBTO): an Integrated Approach to the Evaluation of the Relative Sensitivities of Two Marine Species, *Marine Environmental Research,* 50, 565, 2000a.

428. **Jha, A.N., Hagger, J.A., and Hill, S.J.**, Tributyltin Induces Cytogenetic Damage in the Early Life Stages of the Marine Mussel, Mytilus Edulis, *Environmental and Molecular Mutagenesis,* 35, 343, 2000b.

429. **Larson, D., McDonald, S., Fivizzani, A., Newton, W., and Hamilton, S.**, Effects of the Herbicide Atrazine on Ambystoma Tigrinum Metamorphosis: Duration, Larval Growth, and Hormonal Response, *Physiological Zoology,* 71, 671, 1998.

430. **Allran, J. and Karasov, W.**, Effects of Atrazine and Nitrate on Northern Leopard Frog (Rana Pipiens) Larvae Exposed in the Laboratory From Posthatch Through Metamorphosis, *Environmental Toxicology and Chemistry,* 19, 2850, 2000.

431. **Morgan, M., Scheuerman, P., Bishop, C., and Pyles, R.**, Teratogenic Potential of Atrazine and 2,4-D Using FETAX , *Journal of Toxicology and Environmental Health,* 48, 151, 1996.

432. **Crain, D.A., Guillette, L.J., Rooney, A.A., and Pickford, D.B.**, Alterations in Steroidogenesis in Alligators (Alligator Mississippiensis) Exposed Naturally and Experimentally to Environmental Contaminants, *Environmental Health Perspectives,* 105, 528, 1997.

433. **Crain, D.A., Spiteri, I.D., and Guillette, L.J.**, The Functional and Structural Observations of the Neonatal Reproductive System of Alligators Exposed in Ovo to Atrazine, 2,4-D, or Estradiol, *Toxicology and Industrial Health,* 15, 180, 1999.

434. **Kettle, W., deNoyelles, Jr.f., Heacock, B., and Kadoum, A.**, Diet and Reproductive Success of Bluegill Recovered From Experimental Ponds Treated With Atrazine , *Bulletin of Environmental Contamination and Toxicology,* 38, 47, 1987.

435. **Connor, K., Howell, J., Chen, I., Liu, H., Berhane, K., Sciaretta, C., Safe, S., and Zacharewski, T.**, Failure of Chloro-s-Triazine-Derived Compounds to Induce Estrogen Receptor-Mediated Responses in Vivo and in Vitro, *Fundamental and Applied Toxicology,* 30, 93, 1996.

436. **Tennant, M., Hill, D., Eldridge, J., Wetzel, L., Breckenridge, C., and Stevens, J.**, Possible Antiestrogenic Properties of Chloro-s-Triazines in Rat Uterus, *Journal of Toxicology and Environmental Health* , 43, 183, 1994.

437. **Balaguer, P., Francois, F., Comunale, F., Fenet, H., Boussioux, A., Pons, M., Nicolas, J., and Casellas, C.**, Reporter Cell Lines to Study the Estrogenic Effects of Xenoestrogens, *Science of the Total Environment* , 233, 47, 1999.

438. **Tran, D., Kow, K., McLachlan, J., and Arnold, S.**, The Inhibition of Estrogen Receptor-Mediated Responses by Chloro-S-Triazine-Derived Compounds Is Dependent on Estradiol Concentration in Yeast, *Biochemical and Biphysical Research Communications* , 227, 140, 1996.

439. **Danzo, B.**, Environmental Xenobiotics May Disrupt Normal Endocrine Function by Interfering With the Binding of Physiological Ligands to Steroid Receptors and Binding Proteins, *Environmental Health Perspectives*, 105, 294, 1997.

440. **Cooper, R., Stoker, T., Tyrey, L., Goldman, J., and McElroy, W.**, Atrazine Disrupts the Hypothalamic Control of Pituitary-Ovarian Function, *Toxicological Sciences*, 53, 297, 2000.

441. **McMaster, M.E., Van Der Kraak G.J., Portt C.B., Munkittrick K.R., Sibley P.K., Smith I.R., and Dixon D.G.**, Changes in Hepatic Mixed-Function Oxygenase (MFO) Activity, Plasma Steroid Levels and Age at Maturity of a White Sucker (Catostomus Commersoni) Population Exposed to Belached Kraft Pulp Mill Effluent, *Aquatic Toxicology*, 21, 199, 1991.

442. **McMaster, M.E., Van Der Kraak G.J., and Munkittrick K.R.**, An Epidemiological Evaluation of the Biochemical Basis for Steroid Hormonal Depressions in Fish Exposed to Industrial Wastes, *Journal of Great Lakes Research*, 22, 153, 1996.

443. **McMaster, M.E., Van Der Kraak G.J., and Munkittrick K.R.**, Exposure to Bleached Kraft Pulp Mill Effluent Reduces the Steroid Biosynthetic Capacity of White Sucker Ovarian Follicles, *Comparative Biochemistry and Physiology C*, 112, 169, 1995.

444. **Munkittrick, K.R., McMaster M.E., Portt C.B., Van Der Kraak G.J., Smith I.R., and Dixon D.G.**, Changes in Maturity, Plasma Sex Steroid Levels, Hepatic Mixed-Function Oxygenase Activity, and the Presence of External Lesions in Lake Whitefish (Coregonus Clupleaformis) Exposed to Bleached Kraft Mill Effluent, *Canadian Journal of Fisheries and Aquatic Sciences*, 49, 1560, 1992a.

445. **Munkittrick, K.R., Portt C.B., Van Der Kraak G.J., Smith I.R., and Rokosh D.A.**, Impact of Bleached Kraft Mill Effluent on Population Characteristics, Liver MFO Activity, and Serum Steroid Levels of a Lake Superior White Sucker (Catostomus Commersoni) Population, *Canadian Journal of Fisheries and Aquatic Sciences*, 48, 1371, 1991.

446. **Munkittrick, K.R., McMaster M.E., Portt C.B., Van Der Kraak G.J., Smith I.R., and Dixon D.G.**, Changes in Maturity, Plasma Sex Steroid Levels, Hepatic Mixed-Function Oxygenase Activity, and the Presence of External Lesions in Lake Whitefish (Coregonus Clupleaformis) Exposed to Bleached Kraft Mill Effluent, *Canadian Journal of Fisheries and Aquatic Sciences*, 49, 1560, 1992a.

447. **Munkittrick, K.R., Van Der Kraak, G.J., McMAster, M.E., and Portt, C.B.**, Response of Hepatic MFO Activity and Plasma Sex Steroids to Secondary Treatment of Bleached Kraft Pulp Mill Effluent and Mill Shutdown, *Environmental Toxicology and Chemistry*, 11, 1427, 1992.

448. **Gagnon, M.M., Dodson J.J., Hodson P.V., Van Der Kraak G., and Carey J.H.**, Seasonal Effects of Bleached Kraft Mill Effluent on Reproductive Parameters of White Sucker (Catostomus Commersoni) Populations of the St. Maurice River, Quebec, Canada, *Canadian Journal of Fisheries and Aquatic Sciences*, 51, 337, 1994a.

449. **Munkittrick, K.R., Van Der Kraak G.J., McMaster M.E., Portt C.B., Van DenHeuvel M.R., and Servos M.R.**, Survey of Receiving-Water Environmental Impacts Associated With Discharges From Pulp Mills. 2. Gonad Size, Liver Size, Hepatic EROD Activity and Plasma Sex Steroid Levels in White Sucker, *Environmental Toxicology and Chemistry*, 13, 1089, 1994.

450. **Hodson, P.V., McWhirter, M., Ralph, K., Gray, B., Thivierge, D., Carey, J., Van Der Kraak, G.,**

Whittle, D.M., and Levesque, M.C., Effects of Bleached Kraft Mill Effluent on Fish in the St. Mauruce River, Quebec. *Environmental Toxicology and Chemistry,* 11, 635, 1992.

451. **Gagnon, M.M., Dodson J.J., and Hodson P.V.**, Ability of BKME (Bleached Kraft Mill Effluent) Exposed White Suckers (Catostomus Commersoni) to Synthesize Steroid Hormones, *Comparative Biochemistry and Physiology C,* 107, 265, 1994.

452. **Adams, M.S., Crumby W.D., Greeley M.S., Shugart L.R., and Saylor C.F.**, Responses of Fish Populations and Communities to Pulp Mill Effluents: A Holistic Assessment, *Ecotoxicology and Environmental Safety,* 24, 347, 1992.

453. **Sepulveda, M. S.** "Effects of Paper Mill Effluents on the Health and Reproductive Success of Largemouth Bass (*Micropterus Salmoides*): Field and Laboratory Studies." University of Florida, 2000.

454. **Sandstrom, O.,** *In Situ* Assessments of the Impact of Pulp Mill Effluent on Life-History Variables in Fish, in *Environmental Fate and Effects of Pulp and Paper Mill Effluents.*, Eds., St. Lucie Press, Boca Raton, FL, 1996, 449.

455. **Landner, L., Neilsson, A.H., Sorensen, L., Tarnholm, A., and Viktor, T.**, Short-Term Test for Predicting the Potential of Xenobiotics to Impair Reproductive Success in Fish, *Ecotoxicology and Environmental Safety,* 9, 282, 1985.

456. **Vuorinen, P.J. and Vuorinen, M.**, Effects of Bleached Kraft Mill Effluent on Reproduction of Brown Trout (*Salmo Trutta* L.) on a Restricted Diet. *Finnish Fisheries Research,* 6, 92, 1985.

457. **Tana, J.J.**, Sublethal Effects of Chlorinated Phenols and Resin Acids on Rainbow Trout (Salmo Gairdneri), *Water Science and Technology,* 20, 77, 1988.

458. **Kovacs, T.G., Gibbons, J.S., Tremblay, L.A., O'Conner, B.I., Martel, P.H., and Voss, R.I.**, Effect of a Secondary-Treated Bleached Kraft Pulp Mill Effluent on Aquatic Organisms As Assessed by Short-Term and Long-Term Laboratory Tests, *Ecotoxicology and Environmental Safety,* 31, 7, 1995.

459. **Gagnon, M.M., Bussieres D., Dodson J.J., and Hodson P.V.**, White Sucker (Catostomus Commersoni) Growth and Sexual Maturation in Pulp Mill-Contaminated and Reference Rivers, *Environmental Toxicology and Chemistry,* 14, 317, 1995.

460. **Gagnon, M.M., Dodson J.J., Hodson P.V., Van Der Kraak G., and Carey J.H.**, Seasonal Effects of Bleached Kraft Mill Effluent on Reproductive Parameters of White Sucker (Catostomus Commersoni) Populations of the St. Maurice River, Quebec, Canada, *Canadian Journal of Fisheries and Aquatic Sciences,* 51, 337, 1994a.

461. **McMaster, M.E., Portt, C.B., Munkittrick, K.R., and Dixon, D.G.**, Milt Characteristics, Reproductive Performance, and Larval Survival and Development of White Sucker Exposed to Bleached Kraft Mill Effluent, *Ecotoxicology and Environmental Safety,* 23, 103, 1992.

462. **Swanson, S., Shelast R., Schryder R., Kloepper-Sams P., Marchant T., Kroeber K., Bernstein J., and Owens J.W.**, Fish Populations and Biomarker Responses at a Canadian Bleached Kraft Mill Site, *Tappi Journal,* 75, 139, 1992.

463. **Karås, P., Neuman, E., and Sandström, O.**, Effects of a Pulp Mill Effluent on the Population Dynamics of Perch, Perca Fluviatilis, *Canadian Journal of Fisheries and Aquatic Sciences,* 48, 28, 1991.

464. **Kovacs, T.G., Gibbons, J.S., Martel, P.H., and Voss, R.H.**, Improved Effluent Quality at a Bleached Kraft Mill As Determined by Laboratory Biotests, *Journal of Toxicology and Environmental Health,* 49, 533, 1996.

465. **Kovacs, T.G. and Megraw, S.R.,** Laboratory Responses of Whole Organisms Exposed to Pulp and Paper Mill Effluents: 1991-1994, in *Environmental Fate and Effects of Pulp and Paper Mill Effluents.*, Eds., St. Lucie Press, DelRay Beach, FL, 1996, 459.

466. **Bortone, S.A. and Davis, W.P.**, Fish Intersexuality As Indicator of Environmental Stress, *BioScience,* 44, 165, 1994.

467. **Howell, W.M., Black, D.A., and Bortone, S.A.**, Abnormal Expression of Secondary Sex Characters in a Population of Mosquitofish, *Gambusia Affinis Holbrooki* : Evidence for Environmentally-Induced Masculinization, *Copeia,* 4, 676, 1980.

468. **Bortone, S.A. and Cody, R.P.**, Morphological Masculinization in Poecillid Females From a Paper Mill Effluent Receiving Tributary of the St. John's River, Florida, USA, *Bulletin of Environmental Toxicology and Chemistry,* 63, 150, 1999.

469. **Howell, W.M. and Denton T.E.**, Gonopodial Morphogenesis in Female Mosquitofish, Gambusia Affinis Affinis, Masculinized by Exposure to Degradation Products From Plant Sterols, *Environmental Biology of Fishes,* 24, 43, 1989.

470. **Van Der Kraak, G.J., Munkittrick K.R., McmAster M.E., Portt C.B., and Chang J.P.**, Exposure to Bleached Kraft Pulp Mill Effluent Disrupts the Pituitary-Gonadal Axis of White Sucker at Multiple Sites, *Toxicology and Applied Pharmacology,* 115, 224, 1992.

471. **Janz, D.M., McMaster, M.E., Munkittrick, K.R., and Van Der Kraak, G.**, Elevated Ovarian Follicular Apoptosis and Heat Shock Protein-70 Expression in White Sucker Exposed to Bleached Kraft Pulp Mill Effluent, *Toxicology and Applied Pharmacology,* 147, 391, 1997.

472. **Safe, S.H.,** 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Environmental Antiestrogens: Characterization and Mechanism of Action, in *Endocrine Disruptors. Effects on Male and Female Reproductive Systems.*, Eds., CRC, Boca Raton, FL, 1999, 187.

473. **Van Der Kraak, G., Munkittrick, K.R., McMaster, M.E., and MacLatchy, D.L.,** A Comparison of Bleached Kraft Mill Effluent, 17B-Estradiol, and B-Sitosterol Effects on Reproductive Function in Fish, in *Principles and Processes for Evaluating Endocrine Disruption in Wildlife,* Kendall, R.J., Dickerson, R.L., Giesy, J.P., and Suk, W.P., Eds., SETAC PRESS, Pensacola, FL, 1998, 249.

474. **Mellanen, P., Petanen, T., Lehtimaki, J., Makela, S., Bylund, G., Holmbom, B., Mannila, E., Oikari, A., and Santti, R.**, Wood-Derived Estrogens: Studies in Vitro With Breast Cancer Cell Lines and in Vivo in Trout, *Toxicology and Applied Pharmacology,* 136, 381, 1996.

475. **MacLatchy, D.L. and Van der Kraak G.J.**, The Phytoestrogen B-Sitosterol Alters the Reproductive Endocrine Status of Goldfish, *Toxicology and Applied Pharmacology,* 134, 305, 1995.

476. **Tremblay, L. and Van Der Kraak, G.V.**, Use of a Series of Homologous in Vitro and in Vivo Assays to Evaluate the Endocrine Modulating Actions of B-Sitosterol in Rainbow Trout, *Aquatic Toxicology,* 43, 149, 1998.

477. **Denton, T.E., Howell W.M., Allison J.J., McCollum J., and Marks B.**, Masculinization of Female Mosquitofish by Exposure to Plant Sterols and Mycobacterium Smegmatis, *Bulletin of Environmental Contamination and Toxicology,* 35, 627, 1985.

478. **Tyler, C. and Routledge, E.**, Natural and Anthropogenic Environmental Oestrogens: the Scientific Basis for Risk Assessment. Oestrogenic Effects in Fish in English Rivers With Evidence of Their Causation, *Pure and Applied Chemistry,* 70, 1795, 1998.

479. **Sumpter, J.**, Xenoendocrine Disrupters - Environmental Impacts, *Toxicology Letters,* 103, 337, 1998.

480. **Jobling, S., Nolan, M., Tyler, C., Brighty, G., and Sumpter, J.**, Widespread Sexual Disruption in Wild Fish, *Environmental Science & Technology,* 32, 2498, 1998.

481. **Jobling, S., Sheahan, D., Osborne, J., Matthiessen, P., and Sumpter, J.**, Inhibition of Testicular Growth in Rainbow Trout (Oncorynchus Mykiss) Exposed to Environmental Estrogens, *Environmental Toxicology and Chemistry,* 15, 194, 1996.

482. **Harries, J., Sheahan, D., Jobling, S., Matthiessen, P., Neall, M., Sumpter, J., Taylor, T., and Zaman, N.**, Estrogenic Activity in Five United Kingdom Rivers Detected by Measurement of Vitellogenesis in Caged Male Trout, *Environmental Toxicology and Chemistry,* 16, 534, 1997.

483. **Jobling S, Reynolds T, White R, Parker MG, and Sumpter JP.**, A Variety of Environmentally Persistent Chemicals, Including Some Phthalate Plasticizers, Are Weakly Estrogenic, *Environmental Health Perspectives,* 103, 582, 1995.

484. **Nimrod, A. and Benson, W.**, Environmental Estrogenic Effects of Alkylphenol Ethoxylates, *Critical Reviews in Toxicology,* 26, 335, 1996.

485. **Desbrow, C., Routledge, E., Brighty, G., Sumpter, J., and Waldock, M.**, Identification of Estrogenic Chemicals in STW Effluent. I. Chemical Fractionation and in Vitro Biological Screening, *Environmental Science and Technology,* 32, 1549, 1998.

486. **Purdom, C., Hardiman, P., Bye, V., Eno, N., Tyler, C., and Sumpter, J.**, Estrogenic Effects of Effluents From Sewage Treatment Works, *Chem. Ecol.,* 8, 275, 1994.

487. **Routledge, E., Sheahan, D., Desbrow, C., Brighty, G., Waldock, M., and Sumpter, J.**, Identification of Estrogenic Chemicals in STW Effluent. II. In Vitro Responses in Trout and Roach , *Environmental and Science Technology,* 32, 1559, 1998.

488. **Jobling, S., Sheahan, D., Osborne, J., Matthiessen, P., and Sumpter, J.**, Inhibition of Testicular Growth in Rainbow Trout (Oncorynchus Mykiss) Exposed to Environmental Estrogens, *Environmental Toxicology and Chemistry,* 15, 194, 1996.

489. **Billard, R., Breton, B., and Richard, M.**, On the Inhibitory Effect of Some Steroids on Spermatogenesis in Adult Rainbow Trout (Salmo Gairdneri), *Canadian Journal of Zoology,* 59, 1479, 1981.

490. **Naylor, C.**, Environmental Fate and Safety of Nonylphenol Ethoxylates, *Textile Chemist and Colorist,* 57, 1577, 1985.

491. **Blackburn, M. and Waldock, M.**, Concentrations of Alkylphenols in Rivers and Estuaries in England and Wales, *Water Research,* 29, 1623, 1995.

492. **Christiansen, T., Korsgaard, B., and Jespersen, A.**, Effects of Nonylphenol and 17B-Oestradiol on Vitellogenin Synthesis, Testicular Structure and Cytology in Male Eelpout *Zoarces Viviparus*, *Journal of Experimental Biology,* 201, 179, 1998.

493. **Lech, J.J., Lewis, S.K., and Ren, L.**, In Vivo Estrogenic Activity of Nonylphenol in Rainbow Trout, *Fundamental and Applied Toxicology,* 30, 229, 1996.

494. **Christensen, L., Korsgaard, B., and Bjerregaard, P.**, The Effect of 4-Nonylphenol on the Synthesis of Vitellogenin in the Flounder Platichthys Flesus, *Aquatic Toxicology* , 46, 211, 1999.

495. **Madsen, S., Mathiesen, A., and Korsgaard, B.**, Effects of 17 Beta-Estradiol and 4-Nonylphenol on Smoltification and Vitellogenesis in Atlantic Salmon (Salmo Salar), *Fish Physiology and Biochemistry* , 17, 303, 1997.

496. **Arukwe A, Celius T, Walther BT, and Goksoyr A**
., Effects of Xenoestrogen Treatment on Zona Radiata Protein and Vitellogenin Expression in Atlantic Salmon (Salmo Salar), *Auatic Toxicology* , 49, 159, 2000.

497. **Shioda, T. and Wakabayashi, M.**, Effect of Certain Chemicals on the Reproduction of Medaka (Oryzias Latipes), *Chemosphere,* 40, 239, 2000.

498. **Nimrod, A.C. and Benson, W.H.**, Estrogenic Responses to Xenobiotics in Channel Catfish (Ictalurus Punctatus), *Marine Environmental Research,* 42, 155, 1996.

499. **Gray, M., Teather, K., and Metcalfe, C.**, Reproductive Success and Behavior of Japanese Medaka (Oryzias Latipes) Exposed to 4-Tert-Octylphenol, *Environmental Toxicology and Chemistry,* 18, 2587, 1999.

500. **Kelly, S.A. and Di Giulio, R.T.**, Developmental Toxicity of Estrogenic Alkylphenols in Killifish (Fundulus Heteroclitus), *Environmental Toxicology and Chemistry,* 19, 2564, 2000.

501. **Gimeno, S., Komen, H., Venderbosch, P.W.M., and Bowmer, T.**, Disruption of Sexual Differentiation in

Genetic Male Common Carp (Cyprinus Carpio) Exposed to an Alkylphenol During Different Life Stages, *Environmental Science & Technology,* 31, 2884, 1997.

502. **Dreze, V., Monod, G., Cravedi, J.P., Biagianti-Risbourg, S., and Le Gac, F.**, Effects of 4-Nonylphenol on Sex Differentiation and Puberty in Mosquitofish (Gambusia Holbrooki), *Ecotoxicology,* 9, 93, 2000.

503. **White, R., Jobling, S., Hoare, S., Sumpter, J., and Parker, M.**, Environmentally Persistent Alkylphenolic Compounds Are Estrogenic, *Endocrinology,* 135, 175, 1994a.

504. **Sumpter, J.P. and Jobling S.**, Vitellogenesis As a Biomarker for Estrogenic Contamination of the Aquatic Environment, *Environmental Health Perspectives,* 103, 173, 1995.

505. **Routledge, E. and Sumpter, J.**, Estrogenic Activity of Surfactants and Some of Their Degradation Products Assessed Using a Recombinant Yeast Screen, *Environmental Toxicology and Chemistry* , 15, 241, 1996.

506. **Soto, A.M., Sonnenschein, C., Chung, K.L., Fernandez, M.F., Olea, N., and Olea Serrano, F.**, The E_SCREEN Assay As a Tool to Identify Estrogens: An Update on Estrogenic Environmental Pollutants, *Environmental Health Perspectives,* 103, 113, 1995.

507. **Soto, A., Justicia, H., Wray, J., and Sonnenschein, C.**, P-Nonyl-Phenol: An Estrogenic Xenobiotic Released From "Modified"Polystyrene, *Environmental Health Perspectives* , 92, 167, 1991.

508. **Jobling, S. and Sumpter, J.P.**, Detergent Components in Sewage Effluent Are Weakly Oestrogenic to Fish: An in Vitro Study Using Rainbow Trout (*Oncorhynchus Mykiss*) Hepatocytes, *Aquatic Toxicology,* 27, 361, 1993.

509. **Flouriot, G., Pakdel, F., Ducouret, B., and Valotaire, Y.**, Influence of Xenobiotics on Rainbow Trout Liver Estrogen Receptor and Vitellogenin Gene Expression., *Journal of Molecular Endocrinology,* 15, 143, 1995.

510. **Smeets, J., Rankouhi, T., Nichols, K., Komen, H., Kaminski, N., Giesy, J., and Van den Berg, M.**, In Vitro Vitellogenin Production by Carp (Cyprinus Carpio) Hepatocytes As a Screening Method for Determining (Anti)Estrogenic Activity of Xenobiotics, *Toxicology and Applied Pharmacology,* 157, 68, 1999.

511. **Ren, L., Lewis, S., and Lech, J.**, Effects of Estrogen and Nonylphenol on the Post-Transcriptional Regulation of Vitellogenin Gene Expression, *Chemico-Biological Interactions,* 100, 67, 1996.

512. **Raychoudhury, S., Blake, C., and Millette, C.**, Toxic Effects of Octylphenol on Cultured Spermatogenic Cells and Sertoli Cells, *Toxicology and Applied Pharmacology,* 157, 192, 1999.

513. **Dodds, E. and Lawson, W.**, Molecular Structure in Relation to Oestrogenic Activity Compounds Without a Phenanthrene Nucleus, *Proceedings of the Royal Society of London B,* 125, 222, 1938.

514. **Morrissey, R., George, J., Price, C., Tyl, R., Marr, M., and Kimmel, C.**, The Developmental Toxicity of Bisphenol A in Rats and Mice., *Fundamental and Applied Toxicology* , 8, 571, 1987.

515. **Lutz, I. and Kloas, W.**, Amphibians As a Model to Study Endocrine Disruptors: I. Environmental Pollution and Estrogen Receptor Binding, *Science of the Total Environment,* 225, 49, 1999.

516. **Perez, P., Pulgar, R., Olea-Serrano, F., Villalobos, M., Rivas, A., Metzler, M., Pedraza, V., and Olea, N.**, The Estrogenicity of Bisphenol A-Related Diphenylalkanes With Various Substituents at the Central Carbon and the Hydroxy Groups, *Environmental Health Perspectives,* 106, 167, 1998.

517. **Krishnan, A., Stathis, P., Permuth, S., Tokes, L., and Feldman, D.**, Bisphenol-A: an Estrogenic Substance Is Released From Polycarbonate Flasks During Autoclaving., *Endocrinology,* 132, 2279, 1993.

518. **Olea, N., Pulgar, R., Perez, P., Olea-Serrano, F., Rivas, A., Novillo-Fertrell, A., Pedraza, V., Soto, A., and Sonnenschein, C.**, Estrogenicity of Resin-Based Composites and Sealants Used in Dentistry, *Environmental Health Perspectives* , 104, 298, 1996.

519. **Gaido, K., Leonard, L., Lovell, S., Gould, J., Babai, D., Portier, C., and McDonnell, D.**, Evaluation of Chemicals With Endocrine Modulating Activity in a Yeast-Based Steroid Hormone Receptor Gene Transcription Assay, *Toxicology and Applied Pharmacology* , 143, 205, 1997.

520. **Milligan, S., Khan, O., and Nash, M.**, Competitive Binding of Xenobiotic Oestrogens to Rat Alpha-Fetoprotein and to Sex Steroid Binding Proteins in Human and Rainbow Trout (Oncorhynchus Mykiss) Plasma, *General and Comparative Endocrinology,* 112, 89, 1998.

521. **Shilling, A. and Williams, D.**, Determining Relative Estrogenicity by Quantifying Vitellogenin Induction in Rainbow Trout Liver Slices, *Toxicology and Applied Pharmacology* , 164, 330, 2000.

522. **Islinger, M., Pawlowski, S., Hollert, H., Volkl, A., and Braunbeck, T.**, Measurement of Vitellogenin-MRNA Expression in Primary Cultures of Rainbow Trout Hepatocytes in a Non-Radioactive Dot Blot/RNAse Protection-Assay, *Science of the Total Environment,* 233, 109, 1999.

523. **Celius, T., Haugen, T., Grotmol, T., and Walther, B.**, A Sensitive Żonagenetic Assay for Rapid in Vitro Assessment of Estrogenic Potency of Xenobiotics and Mycotoxins , *Environmental Health Perspectives,* 107, 63, 1999.

524. **Steinmetz, R., Brown, N., Allen, D., Bigsby, R., and Ben, J.**, The Environmental Estrogen Bisphenol A Stimulates Prolactin Release in Vitro and in Vivo, *Endocrinology,* 138, 1780, 1997.

525. **VomSaal, F., Cooke, P., Buchanan, D., Palanza, P., Thayer, K., Nagel, S., Parmigiani, S., and Welshons, W.**, A Physiologically Based Approach to the Study of Bisphenol A and Other Estrogenic Chemicals on the Size of Reproductive Organs, Daily Sperm Production, and Behavior, *Toxicology and Industrial Health,* 14, 239, 1998.

526. **Nagler, J.J., Aysola, P., and Ruby, S.M.**, Effect of Sublethal Pentaclorophenol on Early Oogenesis in Maturing Female Rainbow Trout (Salmo Gairdneri), *Archives of Environmental Contamination and Toxicology,* 15, 549, 1986.

527. **Cleveland, L., Buckler, D., Mayer, F., and Branson, D.**, Toxicity of Three Preparations of Pentachlorophenol to Fathead Minnows -- A Comparative Study, *Environmental Toxicology and Chemistry,* 1, 205, 1982.

528. **Flouriot, G., Pakdel, F., Ducouret, B., and Valotaire, Y.**, Influence of Xenobiotics in Rainbow Trout Liver Estrogen Receptor and Vitellogenin Gene Expression, *Journal of Molecular Endocrinology,* 15, 143, 1995.

529. **Parks, L. and Leblanc, G.**, Reductions in Steroid Hormone Biotransformation/Elimination As a Biomarker of Pentachlorophenol Chronic Toxicity, *Aquatic Toxicology,* 34, 291, 1996.

530. **Mukherjee, D., Guha D., Kumar V., and Chakrabarty S.**, Impairment of Steroidogenesis and Reproduction in Sexually Mature *Cyprinus Carpio* by Phenol and Sulfide Under Laboratory Conditions, *Aquatic Toxicology,* 21, 29, 1991.

531. **Kumar, V. and Mukherjee, D.**, Phenol and Sulfide Induced Changes in the Ovary and Liver of Sexually Maturing Common Carp, Cyprinus Carpio , *Aquatic Toxicology* , 13, 53, 1988.

532. **Slemr, F. and Langer, E.**, Increase in Global Atmospheric Concentrations of Mercury Inferred From Measurements Over the Atlantic Ocean, *Nature,* 355, 434, 1992.

533. **Swain, E., Engstrom, D.R., Brigham, M.E., Henning, T.A., and Brezonik, P.L.**, Increasing Rates of Atmospheric Mercury Deposition in Midcontinental North America, *Science,* 257, 784, 1992.

534. **Livett, E.A.**, Geochemical Monitoring of Atmospheric Heavy-Metal Pollution - Theory and Applications, *Advances in Ecological Research,* 18, 65, 1988.

535. **Zillioux, E.J., Porcella, D.B., and Benoit, J.M.**, Mercury Cycling and Effects in Freshwater Wetland Ecosystems, *Environmental Toxicology and Chemistry,* 12, 2245, 1993.

536. **Spry, D.J. and Wiener J.G.**, Metal Bioavailability and Toxicity to Fish in Low-Alkalinity Lakes: A Critical Review, *Environmental Pollution,* 71, 243, 1991.

537. **Huckabee, J.W., Elwood, J.E., and Hildebrand, S.G.,** Accumulation of Mercury in Freshwater Biota, in *The Biogeochemistry of Mercury in the Environment,* Nriagu, J.O., Eds., Elsevier, Amsterdam, 1979, 277.

538. **Lange, T.R., Royals, H.E., and Connor, L.L.**, Influence of Water Chemistry on Mercury Concentraion in Largemouth Bass From Florida Lakes, *Transactions of the American Fisheries Society,* 122, 74, 1993.

539. **Lange, T.R., Royals, H.E., and Connor, L.L.**, Mercury Accumulation in Largemouth Bass (*Micropterus Salmoides*) in a Florida Lake, *Archives of Environmental Contamination and Toxicology,* 27, 466, 1994.

540. **Bodaly, R.A., Hecky, R.E., and Fudge, R.J.P.**, Increases in Fish Mercury Levels in Lakes Flooded by the Churchill River Diversion, Northern Manitoba, *Canadian Journal of Fisheries and Aquatic Science,* 41, 682, 1984.

541. **Cumbie, P. M.** "Mercury Accumulation in Wildlife on the Southeast." Dissertation, University of Georgia, 1975.

542. **Cumbie, P.M.**, Mercury in Hair of Bobcats and Raccoons, *The Journal of Wildlife Management,* 39, 419, 1975.

543. **Fimreite, N.**, Mercury Contamination of Aquatic Birds in Northwestern Ontario, *Journal of Wildlife Management,* 38, 120, 1974.

544. **Sheffy, T.B. and St.Amant, J.R.**, Mercury Burdens in Furbearers in Wisconsin, *Journal of Wildlife Management,* 46, 1117, 1982.

545. **Khera, K.S.**, Teratogenic and Genetic Effects of Mercury Toxicity, in *The Biogeochemistry of Mercury in the Environment,* Nriagu, N.O., Eds., Elsevier/North Holland Biomedical Press, New York, 1979, 501.

546. **Mohamed, M.K., Burbacher, T.M., and Mottet, N.K.**, Effects of Methyl Mercury on Testicular Functions in *Macaca Fascicularis* Monkeys, *Pharmacology and Toxicology,* 60, 29, 1987.

547. **Burbacher, T.M., Mohamed, M.K., and Mottett, N.K.**, Methylmercury Effects on Reproduction and Offspring Size at Birth, *Reproductive Toxicology,* 1, 267, 1988.

548. **Khera, K.S.**, Reproductive Capability of Male Rats and Mice Treated With Methylmercury, *Toxicology and Applied Pharmacology,* 24, 167, 1973.

549. **Lee, J.H. and Han, D.H.** , Maternal and Fetal Toxicity of Methylmercuric Chloride Administered to Pregnant Fishcher 344 Rats, *Journal of Toxicology and Environmental Health,* 45, 415, 1995.

550. **Hughes, J.A. and Annau, Z.**, Postnatal Behavioral Effects in Mice After Prenatal Exposure to Methylmercury, *Pharmacology, Biochemistry, and Behavior,* 4, 385, 1976.

551. **Fuyuta, M., Fujimoto, T., and Hirata, S.**, Embryotoxic Effects of Methylmercuric Chloride Administered to Mice and Rats During Organogenesis, *Teratology,* 18, 353, 1978.

552. **Fuyuta, M., Jujimoto, T., and Kiyofuji, E.**, Teratogenic Effects of a Single Oral Administration of Methylmercuric Chloride in Mice, *Acta Anatomy (Basel),* 104, 356, 1979.

553. **Hirano, M., Mitsumori, K., Maita, K., and Shirasu, Y.**, Further Carcinogenicity Study on Methylmercury Chloride in ICR Mice, *Nippon Juigaku Zasshi (Japanese Journal of Veternary Science,* 48, 127, 1986.

554. **Mitsumori, K., Hirano, M., Ueda, H., Maita, K., and Shirasu, Y.**, Chronic Toxicity and Carcinogenicity of Methylmercury Chloride in B6C3F1 Mice, *Fundamental and Applied Toxicology,* 14, 179, 1990.

555. **Inouye, M. and Kajiwara, Y.**, Developmental Disturbances of the Fetal Brain in Guinea Pigs Caused by Methymercury, *Archives of Toxicology,* 62, 15, 1988.

556. **National Research Council**, *Toxicological Effects of Methylmercury* , National Academy Press, Washington, D. C., 2000,
<25 Page(s)>.

557. **Roelke, M. E., D. P. Schultz, C. F. Facemire, S. F. Sundlof, and H. E. Royals.** "Mercury Contamination in Florida Panthers." Florida Panther Interagency Committee, 1991.

558. **Wolfe, M.F., Schwarzbach, S., and Sulaiman, R.A.**, Effects of Mercury on Wildlife: a Comprehensive Review, *Environmental Toxicology and Chemistry,* 17, 146, 1998.

559. **Von Burg, R. and Greenwood, M.R.,** Mercury, in *Metals and Their Compounds in the Environment,* Merian, E., Eds., VCH, Weinheim, Germany, 1991.

560. **Barr, J.F.**, Population Dynamics of the Common Loon (Gavia Immer) Associated With Mercury-Contaminated Waters in Northwestern Ontario, *CANADIAN WILDLIFE SERVICE OCCASIONAL PAPER No,* 56, 1, 1986.

561. **Wiemeyer, S.N., Lamont, T.G., Bunck, C.M., Sindelar, C.R., Gramlich, F.J., Fraser, J.D., and Byrd, M.A.** , Organochlorine Pesticide, Polychlorobiphenyl, and Mercury Residues in Bald Eagle Eggs - 1969-79 - and Their Relationships to Shell Thinning and Reproduction, *Archives of Environmental Contamination and Toxicology,* 13, 529, 1984.

562. **Furness, R.W., Muirhead S.J., and Woodburn M.**, Using Bird Feathers to Measure Mercury in the Environment: Relationships Between Mercury Content and Moult, *Marine Pollution Bulletin,* 17, 27, 1986.

563. **Furness, R.W. and Hutton, M.**, Pollutants and Impaired Breeding of Great Skuas Catharacta-Skua in Britain, *Ibis,* 122, 88, 1980.

564. **Finley, M.T. and Stendell R.C.**, Survival and Reproductive Success of Black Ducks Fed Methyl Mercury, *Environmental Pollution,* 16, 51, 1978.

565. **Newton, I. and Haas, M.B.**, Pollutants in Merlin Eggs and Their Effects on Breeding, *Br Birds,* 81, 258, 1988.

566. **Heinz, G.H.**, Methylmercury: Reproductive and Behavioral Effects on Three Generations of Mallard Ducks, *Journal of Wildlife Management,* 43, 394, 1979.

567. **Heinz, G.**, Effects of Low Dietary Levels of Methyl Mercury on Mallard Reproduction, *Bulletin of*

Environmental Contamination and Toxicology, 11, 386, 1974.

568. **Zoll, C., Saouter, E., Boudou, A., Ribeyre, F., and Jaylet, A.**, Genotoxicity and Bioaccumulation of Methyl Mercury and Mercuryic Chloride *in Vivo* in the Newt *Pleurodeles Waltl, Mutagenesis,* 3, 337, 1988.

569. **Kanamadi, R.D. and Saidapur, K.S.**, Effects of Exposure to Sublethal Mercuric Chloride on the Testis and Fat Body of the Frog *Rana Cyanophlyctis, Journal of Herpetology,* 26, 499, 1992.

570. **Power, T., K. L. Clark, and A. Harnfenist.** "A Review and Evaluation of the Amphibian Toxicological Literature." Canadian Wildlife Service, 1989.

571. **Ide, C., Jelaso, A., and Austin, C.,** Effects of Methylmercury Chloride on Development of the Frog *Xenopus Laevis*, in *Proceedings, Thirteenth International Neurotoxicology Conference on Developmental Neurotoxicity of Endocrine Disrupters.*, Eds., 1995, pp 763.

572. **Friedmann, A.S., Watzin, M.C., Brinck-Johnson, T., and Leiter, J.C.**, Low Levels of Dietary Methylmercury Inhibit Growth and Gonadal Development in Juvenile Walleye (*Stizostedion Vitreum*), *Aquatic Toxicology,* 35, 265, 1996.

573. **Verma, S.R. and Tonk, I.P.**, Effect of Sublethal Concentrations of Mercury on the Composition of Liver, Muscles and Ovary of Notopterus- Notopterus, *Water Air and Soil Pollution,* 20, 287, 1983.

574. **Billard, R. and Roubaud, P.**, The Effect of Metals and Cyanide on Fertilization in Rainbow- Trout (Salmo-Gairdneri), *Water Research,* 19, 209, 1985.

575. **Khan, A.T. and Weis, J.S.**, Effects of Methylmercury on Sperm and Egg Viability of 2 Populations of Killifish (Fundulus-Heteroclitus), *Archives of Environmental Contamination and Toxicology,* 16, 499, 1987.

576. **Arnold, Beverly Schleppi.** "Distribution of Mercury Within Different Trophic Levels of the Okefenokee Swamp, Within Tissues of Top Level Predators, and Reproductive Effects of Methyl Mercury in the Nile Tilapia (Oreochromis Niloticus) ." University of Georgia, 2000.

577. **Wester, P.W.**, Histopathological Effects of Environmental-Pollutants Beta-Hch and Methyl Mercury on Reproductive-Organs in Fresh-Water Fish, *Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology,* 100, 237, 1991.

578. **Rom, W.N.**, Effects of Lead on Female and Reproduction, *Mount Sinai Journal of Medicine,* 43, 542, 1976.

579. **Lee, I.P.,** Effects of Environmental Metals on Male Reproduction, in Clarkson, T.W., Nordberg, G.F., and Sager, P.R., Eds., Plenum Press, New York, 1983, 253.

580. **Hildebrand, D.C., Der, H., Griffin, W. and Fahim, F.S.**, Effect of Lead Acetate on Reproduction, *American Journal of Obstetrics and Gynecology,* 115, 1058, 1973.

581. **Barltop, D.,** Transfer of Lead to the Human Fetus, in *Mineral Metabolism in Pediatrics,* Barltop, D. and Barland, W.L., Eds., Davis, Philadelphia, 1969, 135.

582. **Davis, J.M.**, Risk Assessment of the Developmental Neurotoxicity of Lead, *Neurotoxicology,* 11, 285, 1990.

583. **Beach, J.R. and Henning, S.J.**, The Distribution of Lead in Milk and the Fate of Milk Lead in the Gastrointestinal-Tract of Suckling Rats, *Pediatric Research,* 23, 58, 1988.

584. **Hogstedt, C., Hane, M., Agrell, A., and Bodin, L.**, Neuropsychological Test-Results and Symptoms Among Workers With Well-Defined Long-Term Exposure to Lead, *British Journal of Industrial Medicine,* 40, 99, 1983.

585. **Pocock, S.J., Shaper, A.G., Ashby, D., Delves, H.T., and Clayton, B.E.**, The Relationship Between Blood Lead, Blood-Pressure, Stroke, and Heart-Attacks in Middle-Aged British Men, *Environmental Health Perspectives,* 78, 23, 1988.

586. **Davis, J.M. and Svendsgaard, D.J.**, Lead and Child-Development, *Nature,* 329, 297, 1987.

587. **Davidson, W.R. and Nettles, V.F.**, *Field Mahual of Wildlife Diseases in the Southeastern United States* , Southeastern Cooperative Wildlife Disease Stude, Athens, GA, 1997, <25 Page(s)>.

588. **Burger, J., C. Carruth-Hinchey, E. Ondroff, M. McMahon, J. W. Gibbons, and M. Gochfeld**. "Effects of Lead on Behavior, Growth, and Survival of Hatchling Slider Turtles.", 495-502. 1998.

589. **Horne, M. T., and W. A. Dunson**. "Toxicity of Metals and Low PH to Embryos and Larvae of the Jerrerson Salamander, Ampystoma-Jeffersonianum.", 110-114. 1995.

590. **Reichert, W.L., Federighi, D.A., and Malins, D.C.**, Uptake and Metabolism of Lead and Cadmium in Coho Salmon (Oncorhynchus-Kisutch), *Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology,* 63, 229, 1979.

591. **Tulasi, S.J., Reddy, P.U.M., and Rao, J.V.R.**, Effects of Lead on the Spawning Potential of the Fresh-Water Fish, Anabas-Testudineus, *Bulletin of Environmental Contamination and Toxicology,* 43, 858, 1989.

592. **Panigrahi, A., Dasmahapatra, A.K., and Medda, A.K.**, Effect of Lead, Zinc, Mercury, and Copper With and Without Estrogen on Serum Vitellogenin Level in Magur Fish (*Clarias Batrachus* L.)., *Gegenbaurs Morphologisches Jahrbuch,* 136, 775, 1990.

593. **Kumar, S. and Pant, S.C.**, Comparative Effects of the Sublethal Poisoning of Zinc, Copper and Lead on the Gonads of the Teleost Puntius-Conchonius Ham, *Toxicology Letters,* 23, 189, 1984.

594. **Srivastava, A.K.**, Changes Induced by Lead in Fish Testis, *Journal of Environmental Biology,* 8, 329, 1987.

595. **Sehgal, R., Tomar, V., and Pandey, A.K.**, Comparative Effects of 2 Heavy Metallic Salts on the Testis of Viviparous Teleost, Lebistes-Reticulatus (Peters), *Journal of Environmental Biology,* 5, 185, 1984.

596. **Myint, U.M. and Tyler, P.A.**, Effects of Temperature, Nutritive and Metal Stressors on the Reproductive Biology of *Mytilis Edulis*, *Marine Biology,* 67, 209, 1982.

597. **Munkittrick, K.R. and Dixon, D.G.**, Effects of Natural Exposure to Copper and Zinc on Egg Size and Larval Copper Tolerance in White Sucker (Catostomus-Commersoni), *Ecotoxicology and Environmental Safety,* 18, 15, 1989.

598. **Mckim, J.M. and Benoit, D.A.**, Effects of Long-Term Exposures to Copper on Survival, Growth, and Reproduction of Brook Trout (Salvelinus-Fontinalis), *Journal of the Fisheries Research Board of Canada,* 28, 655-&, 1971.

599. **Anderson, B.S., Middaugh, D.P., Hunt, J.W., and Turpen, S.L.**, Copper Toxicity to Sperm, Embryos and Larvae of Topsmelt Atherinops-Affinis, With Notes on Induced Spawning, *Marine Environmental Research,* 31, 17, 1991.

600. **Busacker, G.P. and Chavin, W.**, Uptake, Distribution and Biological Half-Life of Cd-109 in Tissues of Carassius-Auratus L, *American Zoologist,* 17, 865, 1977.

601. **Joensen, J.E. and Korsgaard, B.**, Uptake, Time-Course Distribution and Elimination of Cadmium in Embryos and Tissues of the Pregnant Zoarces-Viviparus (L) After Intraovarian Loading, *Journal of Fish Biology,* 28, 61, 1986.

602. **Ghosh, P. and Thomas, P.**, Binding of Metals to Red Drum Vitellogenin and Incorporation into Oocytes, *Marine Environmental Research,* 39, 165, 1995.

603. **Shackley, S.E., King, P.E., and Gordon, S.M.**, Vitellogenesis and Trace Metals in a Marine Teleost, *Journal of Fish Biology,* 18, 349, 1981.

604. **White, D.H. and Finley, M.T.**, Uptake and Retention of Dietary Cadmium in Mallard Ducks, *Environmental Research,* 17, 53, 1978.

605. **Sell, J.L.**, Cadmium and the Laying Hen: Apparent Absorption, Tissue Distribution, and Virtual Absence of Transfer into Eggs, *Poultry Science,* 54, 1674, 1975.

606. **Furness, R.W.,** Cadmium in Birds, in *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations,* Beyer, W.N., Heinz, G.H., and Redmon-Norwood, A.W., Eds., Lewis Publishers, Boca Raton, Fl, 1996, 389.

607. **Richardson, M.E. and Fox, M.R.S.**, Dietary Cadmium and Enteropathy in the Japanese Quail - Histochemical and Ultrastructural Studies, *Laboratory Investigations,* 31, 722, 1975.

608. **Kotyzova, D. and Sundeman, F.W.**, Maternal Exposure to Cd(II) Causes Malformations of Xenopus Laevis Embryos, *Annals of Clinical and Laboratory Science,* 28, 224, 1998.

609. **Herkovits, J., Cardellini, P., Pavanati, C., and PerezColl, C.S.**, Susceptibility of Early Life Stages of Xenopus Laevis to Cadmium, *Environmenatl Toxicology and Chemistry,* 16, 312, 1997.

610. **Haux, C., Bjornsson, B.T., Förlin, L., Larsson, A., and Deftos, L.J.**, Influence of Cadmium Exposure on Plasma Calcium, Vitellogenin and Calcitonin in Vitellogenic Rainbow Trout, *Marine Environmental Research,* 24, 199, 1988.

611. **Sangalang, G.B. and O'Halloran, M.J.**, Cadmium-Induced Testicular Injury and Alterations of Androgen Synthesis in Brook Trout, *Nature,* 240, 460, 1972.

612. **Victor, B., Mahalingam, S., and Sarojini, R.**, Toxicity of Mercury and Cadmium on Oocyte Differentiation and Vitellogenesis of the Teleost, *Lepidocephalichtyhs Thermalis* (Bleeker)., *Journal of Environmental Biology,* 7, 209, 1986.

613. **Pereira, J.J., Ziskowski, J., Mercaldo-Allen, R., Kuropat, C., Luedke, D., and Gould, E.**, Vitellogenin in Winter Flounder (*Pleuronectes Americanus*) From Long Island Sound and Boston Harbor., *Estuaries Estudo,* 15, 289, 1992.

614. **Povlsen, A.F., Korsgaard, B., and Bjerregaard, P.**, The Effect of Cadmium on Vitellogenin Metabolism in Estradiol-Induced Flounder (*Platichtys Flesus* L.) Males and Females, *Aquatic Toxicology,* 17, 253, 1990.

615. **Sangalang, G.B. and Freeman, H.C.**, Effect of Sublethal Cadmium on Maturation and Testosterone, and 11-Ketotestosterone Production in Brook Trout (*Salvelinus Fontinalis*)., *Biology of Reproduction,* 11, 429, 1974.

616. **Thomas, P.**, Effects of Aroclor 1254 and Cadmium on Reproductive Endocrine Function and Ovarian Growth in Atlantic Croaker, *Marine Environmental Research,* 28, 499, 1989.

617. **Kime, D.E.**, The Effect of Cadmium on Steroidogenesis by Testes of the Rainbow-Trout, Salmo-Gairdneri, *Toxicology Letters,* 22, 83, 1984.

618. **Pundir, R. and Saxena, A.B.**, Seasonal-Changes in the Testes of Fish Puntius-Ticto, and Their Relation to Heavy-Metal Toxicity, *Bulletin of Environmental Contamination and Toxicology,* 45, 288, 1990.

619. **Kumari, M. and Dutt, N.H.G.**, Cadmium-Induced Histomorphological Changes in the Testis and Pituitary Gonadotropic Hormone Secreting Cells of the Cyprinid Puntius-Sarana, *Bollettino Di Zoologia,* 58, 71, 1991.

620. **Hatakeyama, S. and Yasuno, M.**, Chronic Effects of Cd on the Reproduction of the Guppy (Poecilia-Reticulata) Through Cd-Accumulated Midge Larvae (Chironomus-Yoshimatsui), *Ecotoxicology and Environmental Safety,* 14, 191, 1987.

621. **Speranza, A.W., Seeley, R.J., Seeley, V.A., and Perlmutter, A.**, Effect of Sublethal Concentrations of Zinc on Reproduction in Zebrafish, Brachydanio-Rerio Hamilton-Buchanan, *Environmental Pollution,* 12, 217, 1977.

622. **Kumar, S. and Pant, S.C.**, Comparative Effects of the Sublethal Poisoning of Zinc, Copper and Lead on the Gonads of the Teleost Puntius-Conchonius Ham, *Toxicology Letters,* 23, 189, 1984.

623. **Munkittrick, K.R. and Dixon, D.G.**, Use of White Sucker (*Catostomus Commersoni*) Populations to Assess the Health of Aquatic Ecosystems Exposed to Low-Level Contaminant Stress, *Canadian Journal of Fisheries and Aquatic Sciences,* 46, 1455, 1989.

624. **Ohlendorf, H.M., Hoffman, D.J., Saiki, M.K., and Aldrich, T.W.**, Embryonic Mortality and Abnormalities of Aquatic Birds - Apparent Impacts of Selenium From Irrigation Drainwater, *Science of the Total Environment,* 52, 49, 1986.

625. **Ohlendorf, H.M., Kilness, A.W., Simmons, J.L., Stroud, R.K., Hoffman, D.J., and Moore, J.F.**, Selenium Toxicosis in Wild Aquatic Birds, *Journal of Toxicology and Environmental Health,* 24, 67, 1988.

626. **Lemly, A.D.,** Selemium in Aquatic Organisms, in *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations,* Beyer, W.N., Heinz, G.H., and Redmon-Norwood, A.W., Eds., Lewis Publishers, Boca Raton, 1996, 427.

627. **Heinz, G.H.,** Selenium in Birds, in *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations,* Beyer, W.N., Heinz, G.H., and Redmon-Norwood, A.W., Eds., Lewis Publishers, Boca Raton, 1996, 447.

628. **Heinz, G.H., Hoffman, D.J., and Gold, L.G.**, Impaired Reproduction of Mallards Fed an Organic Form of Selenium, *Journal of Wildlife Management,* 53, 418, 1989.

629. **Heinz, G.H., Hoffman, D.J., Krynitsky, A.J., and Weller, D.M.G.**, Reproduction in Mallards Fed Selenium, *Environmental Toxicology and Chemistry,* 6, 423, 1987.

630. **Heinz, G.H.,** Selenium in Birds, in *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations,* Beyer, W.N., Heinz, G.H., and Redmon-Norwood, A.W., Eds., Lewis Publishers, Boca Raton, 1996, 447.

631. **Gutleb, A.C., Appelman, J., Bronkhorst, M., Van Den Berg, J.H.J., and Murk, A.J.**, Effects of Oral Exposure to Polychlorinated Biphenyls (Pcbs) on the Development and Metamorphosis of Two Amphibian Species (Xenopus Laevis and Rana Temporaria), *Science of the Total Environment,* 262, 147, 2000.

632. **Mac, M.J. and Schwartz, T.R.**, Investigations into the Effects of PCB Congeners on Reproduction in Lake Trout From the Great Lakes, *Chemosphere,* 25, 189, 1992.

633. **Allen, J.R. and Lambrecht, L.**, Response of Rhesus Monkeys to Polybrominated Biphenyls, *Toxicology and Applied Pharmacology,* 45, 340, 1978.

634. **Beaudoin, A.R.**, Teratogencity of Polybrominated Biphenyls in Rats, *Environmenatl Research,* 14, 81, 1977.

635. **Lockie, J.D., Ratcliff.da, and Balharry, R.**, Breeding Success and Organo-Chlorine Residues in Golden Eagles in West Scotland, *Journal of Applied Ecology,* 6, 381-&, 1969.

636. **Henny, C.J., Blus, L.J., and Stafford, C.J.**, Effects of Heptachlor on American Kestrels in the Columbia Basin, Oregon, *Journal of Wildlife Management,* 47, 1080, 1983.

637. **Willingham, E. and Crews, D.**, Sex Reversal Effects of Environmentally Relevant Xenobiotic Concentrations on the Red-Eared Slider Turtle, a Species With Temperature-Dependent Sex Determination, *General and Comparative Endocrinology,* 113, 429, 1999.

638. **Smith, R.M. and Cole, C.F.**, Effects of Egg Concentrations of Ddt and Dieldrin on Development in Winter Flounder (Pseudopleuronectes-Americanus), *Journal of the Fisheries Research Board of Canada,* 30, 1894, 1973.

639. **Kulshrestha, S.K. and Arora, N.**, Effect of Sublethal Doses of Carbaryl and Endosulfan on the Skin of Channa-Striatus Bl, *Journal of Environmental Biology*, 5, 141, 1984.

640. **Ratcliff, D.A.**, Decrease in Eggshell Weight in Certain Birds of Prey, *Nature*, 215, 208-&, 1967.

641. **Blus, L.J. and Wiemeyer, S.N.H.C.J.**, Organochlorine Pesticides, in *Noninfectious Diseases of Wildlife*, Fairbrother A., Locke, L.N., and Hoff, G.L., Eds., Iowa State University, Ames, IA, 1996, 61.

642. **Willingham, E. and Crews, D.**, Sex Reversal Effects of Environmentally Relevant Xenobiotic Concentrations on the Red-Eared Slider Turtle, a Species With Temperature-Dependent Sex Determination, *General and Comparative Endocrinology*, 113, 429, 1999.

643. **Gross, T.S., Guillette L.J., Percival H.F., Masson G.R., Matter J.M., and Woodward A.R.**, Contaminant-Induced Reproductive Anomalies in Florida, *Comparative Pathology Bulletin*, 26, 1, 1994.

644. **Chadwick, R.W., Cooper, R.L., Chang, J., Rehnberg, G.L., and McElroy, W.K.**, Possible Antiestrogenic Activity of Lindane in Female Rats, *Journal of Biochemical Toxicology*, 3, 1988.

645. **Whitehead, C.C., Downie, J.N., and Phillips, J.A.**, BHC Not Found to Reduce Shell Quality of Hen's Eggs, *Nature*, 239, 411, 1972.

646. **Chakravorty, S. and Lahiri, P.**, Effect of Lindane on Eggshell Characteristics and Calcium Level in the Domestic Duck, *Journal of Biochemical Toxicology*, 3, 147, 1986.

647. **Singh, P.B. and Singh, T.P.**, Impact of G-BHC on Sex Steroid Levels and Their Modulation by Ovine Luteinizing Hormone-Releasing Hormone and Mystus Gonadotropin in the Freshwater Catfish, *Heteropneustes Fossilis*, *Aquatic Toxicology*, 21, 93, 1991.

648. **Kelce, W.R., Monosson, M.P., Gamcsik, M.P., Laws, S.C., and Gray Jr., L.E.**, Environmental Hormone Disruptors: Evidence That Vinclozolin Developmental Toxicity Is Mediated by Antiandrogenic Metabolites, *Toxicology and Applied Pharmacology*, 126, 276, 1994.

649. **Wannemacher, R., Rebstock, A., Kulzer, E., Schrenk, D., and Bock, K.W.**, Effects of 2,3,7,8-Tetrachlorodibenzo-Dioxin on Reproduction and Oogenesis in Zebrafish (*Brachydanio Rerio*), *Chemosphere*, 24, 1361, 1992.

650. **Bortone, S.A. and Davis, W.P.**, Fish Intersexuality As Indicator of Environmental Stress: Monitoring Fish Reproductive Systems Can Serve to Alert Humans to Potential Harm, *Bioscience*, 44, 165, 1994.

651. **Barr, J. F**. "Population Dynamics of the Common Loon (*Gavia Immer*) Associated With Mercury-Contaminated Waters in North-Western Ontario." *Occasional Paper 56*, Canadian Wildlife Service, Ottawa, ON, 1986.

652. **Kanamadi, R.D. and Saidapur, K.S.**, Effects of Exposure to Sublethal Mercuric Chloride on the Testis and Fat Body of the Frog *Rana Cyanophlyctis*, *Journal of Herpetology*, 26, 499, 1992.

653. **Friedmann, A.S., Watzin, M.C., Brinck-Johnson, T., and Leiter, J.C.**, Low Levels of Dietary Methylmercury Inhibit Growth and Gonadal Development in Juvenile Walleye (*Stizostedion Vitreum*), *Aquatic Toxicology*, 35, 265, 1996.