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Endocrine Disruptor: Its Role and Remedy

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Endocrine disruptor interferes with the biological functions of natural hormones in the body which is responsible for the maintenance of homeostasis, reproduction, development, and/or behavior. A number of chemicals in the environment may disrupt the endocrine systems of aquatic and wildlife and have been shown to disrupt female reproductive function throughout the life span. Certain endocrine-disrupting chemicals can substantially reduce some animal populations and there can be extreme differences in the susceptibility between species to these chemicals. A variety of test methods are available but it is not known which one(s) is the best to determine the effects of endocrine-disrupting chemicals.

Key words: Endocrine, disruptor, effect

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Introduction

An environmental endocrine disruptor is defined as an exogenous agent that interferes with the synthesis, secretion, transport, binding, action or elimination of natural hormones in the body and that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior. For the purpose of this document, the term "endocrine disruptor" will be used as synonymous with hormone disruptor. Of importance here is the concept that endocrine disruptors encompass more than just environmental estrogens and include any agent that adversely affects any aspect of the entire endocrine system. Endocrine disruptors are usually either natural products or synthetic chemicals that mimic, enhance (an agonist), or inhibit (an antagonist) the action of hormones.

The potential role of environmental endocrine disruption in the induction of breast, testicular and prostate cancers, as well as endometriosis, is evaluated. The inter-relationship of the endocrine and immune system is documented. Hormones are natural, secretary products of endocrine glands (ductless glands that discharge directly into the blood stream). Hormones travel in the blood in very small concentrations and bind to specific cell sites called receptors in distant target tissues and organs where they exert their effects on development, growth and reproduction in addition to other body functions. The endocrine system is one of at least three important, integrating and regulatory systems in humans and other animals while the other two are the nervous and immune systems. Hormones influence important regulatory, developmental, growth and homeostatic mechanisms, such as reproductive structure and function; maintenance of normal levels of glucose and ions in blood; control of general body metabolism; blood pressure; other glandular, muscle and nervous system functions. Some of the major endocrine glands include the pituitary, thyroid, pancreas, adrenal, and the male and female gonads (testes and ovaries).

A variety of chemicals have been shown to disrupt female reproductive function in laboratory animal and humans throughout the life span (e.g., diethylstilbestrol). These effects include the disruption of normal sexual differentiation, ovarian function (i.e., follicular growth, ovulation, corpus lutetium formation and maintenance), fertilization, implantation and pregnancy. Only a few agents are associated with direct interference with the endocrine reproductive axis. Examples are those with estrogenic activity or the potential to interact with the aryl hydrocarbon (Ah) receptor. Exposure to toxicants during development is of particular concern because many feedback mechanisms functioning in the adult are absent and adverse effects may be noted at doses lower than those observed in the adult. Endometriosis is a painful reproductive and immunologic disease of women characterized by aberrant location of uterine endometrial cells, the etiology of this disease is unknown (Birnbaum, 1994) suggested a link between dioxin exposure and the development of endometriosis in rhesus monkeys. The severity of this lesion was dependent on the dose administered. Birnbaum (1994) reported the hypothesis that serum dioxin concentrations have an association with human endometriosis. No statistically significant correlations between disease severity and serum levels of halogenated aromatic hydrocarbons were found. These preliminary data, admittedly on a limited population, suggested that serum dioxin concentrations may not be related to human endometriosis.

Human breast cancer is a major health problem in the world. While considerable information is available on risk factors for human breast cancer, the mechanisms of mammary gland carcinogenesis and the precise role played by chemical carcinogens, physical and biological agents, varied life styles, genetic susceptibility and developmental exposures have yet to be elucidated. It has been hypothesized that exposure to organochlorines, some pesticides, and/or polyaromatic hydrocarbons might play a role in the etiology of mammary gland neoplasms via an endocrine disruption pathway, perhaps via an estrogen-mimetic route or alternate estrogen pathways. Investigators began expressing their concern for estrogenic effects of environmental xenobiotic chemicals more than 25 years ago (McLachlan, 1985; Hertz, 1985; Richardson and Bowron, 1985). Within the past 4 years, this concern has become focused and intensified (Rolland *et al*., 1995; McLachlan and Korach, 1995; Kavlock *et al*., 1996; Ankley *et al*., 1996). Attention has been called to the potential hazards that some chemicals may have on human health and ecological well being (breast and reproductive tract cancers, reduced male fertility, abnormality in sexual development, etc.) (Makela *et al*., 1994; Sharpe and Skakkebaek, 1993; Wolff *et al*., 1993; Colborn *et al*., 1996). There has been a considerable controversy over the report that human sperm counts have decreased over the past 50 years.

Clear evidence exists that in utero exposure to certain potent synthetic estrogens such as DES has an adverse reproductive effect in the children of women treated with it during pregnancy and that a rare adenocarcinoma of the vagina was seen some 20 years later in the daughters (Herbst *et al*., 1971). In female rats of the AEI strain, which has a low incidence of spontaneous mammary tumors both prenatal and postnatal exposure to DES increased numbers of mammary tumors (Rothschild *et al*., 1987). Male rats treated from gestational day 14 to postnatal day 3 with the antiandrogenic fungicide vinclozolin exhibt varied reproductive dysfunction as adults (Gray *et al*., 1994). Caged male rainbow trout exposed to effluent from 15 different sewage treatment facilities expressed elevated concentrations of vitellogenin, an estrogen-induced yolk protein precursor (Purdom *et al*., 1994). Furthermore, there is an ample evidence that the pesticide DDT (1,1,1-trichloro-2,2-bis [4-chl orophenyl]ethane) now banned, and its metabolites cause a dwindling bird population due to testicular feminization of male embryos leading to abnormal sex ratios of adult Western gulls in the 1960s (Fry and Toone, 1981).

Chemically, hormones are glycoproteins, polypeptides, peptides, steroids, modified amino acids, catechol amine, prostaglandins and retinoic acid. They are transported in blood at very low concentrations (ng or pg ml^{-1}) in the free state or attached to carrier proteins. They bind to specific cell surfaces or 12 nuclear receptors and exert important regulatory, growth or homeostatic effects. Steroid and thyroid hormones, bound to their protein receptors, regulate gene activity (expression) as DNA transcription factors; protein and peptide hormones function by transmitting a signal (intracellular second messenger) to regulate ion channels or enzymes. The secreted hormones helps to regulate general body growth and metabolism, other endocrine organs and reproductive function. Some target organs and tissues under endocrine control include the mammary glands, bone, muscle, the nervous system and the male and female reproductive organs. In addition to the classical hormones found in higher vertebrates, including humans, there are hormones in invertebrates (e.g., ecdysone) and plants (e.g., auxins). Consequently, when environmental endocrine disruptors mimic or interfere with the action of endogenous hormones, they have the potential of influencing human health and exerting significant ecological effects globally. Under some circumstances, endocrine

disruptor may act as hypertrophic (stimulatory) agents and tumor promoters. Dose, body burden, timing and duration of exposure at critical periods of life are important considerations for assessing adverse effects of an endocrine disruptor. Effects may be reversible or irreversible, immediate (acute) or latent and not expressed for a period of time.

The endocrine system includes a number of central nervous system (CNS)-pituitary-target organ feedback pathways involved in regulating a multitude of bodily functions and maintaining homeostasis. As such, there are potentially several target organ sites at which a given environmental agent could disrupt endocrine function. Furthermore, because of the complexity of the cellular processes involved in hormonal communication, any of these loci could be involved mechanistically in a toxicant's endocrine-related effect. Thus, impaired hormonal control could occur as a consequence of altered hormone: synthesis, storage/release, transport/clearance, receptor recognition/binding, or post-receptor responses.

Effects on Aquatic and Wild life: There is an increasing evidence that a number of chemicals in the environment may disrupt the endocrine systems of aquatic and wildlife. This includes both manmade chemicals (xenobiotics) and chemicals that occur naturally in plants such as phytoestrogens.

Synthetic Chemicals (Xenobiotics): Many synthetic chemicals have been labeled as suspected environmental endocrine disruptors and are addressed briefly below.

These include alkylphenols, bisphenol-A, 2,3,7,8- TCDD, 2,3,7,8-tetrachlorodibenzo-furan (TCDF), polychlorinated biphenyl (PCBs), and some pesticides. Some of the chemicals thought to be environmental endocrine disruptors are in commerce today; however, many other xenobiotics have been prohibited previously from use because of their adverse effects on human health and the environment. Some of these xenobiotic chemicals not in use today is persist in the environment. They are transported and deposited via atmospheric transport from other parts of the world that still use them or from previous environmental contamination (Geisy *et al.*, 1994).

Environmental residues of some xenobiotic compounds have decreased after these chemicals were banned or canceled, but many others have leveled off because of physical properties that cause them to accumulate in sediments, be re-released to the aquatic environment, and accumulate in the tissues of organisms. Purdom *et al.* (1994) suggested that alkylphenol-polyethoxylates (APE), originating from the biodegradation of surfactant and detergents during sewage treatment, and ethynylestradiol, originating from pharmaceutical use, are the two most likely sources of the estrogenic substances present in sewage effluent. Alkylphenols, such as nonylphenol, are commonly used as antioxidants and also are degradates of the biodegradation of a family of nonionic surfactant (such as APE) during sewage treatment (Jobling and Sumpter, 1993). Nonylphenol and other alkyl phenols have been reported to leach from plastics used in food processing and packaging, such as food grade polyvinyl chloride (Junk *et al.*, 1974; Brotons *et al.*, 1995). In the development of a screening assay for estrogenic compounds, nonylphenol was discovered to leach from polystyrene laboratory ware (Soto *et al.*, 1991) and bisphenol-A was released from plastic ware during autoclaving (Krishnan *et al.*, 1993). TCDD and TCDF also suspected of being environmental endocrine disruptors. They are byproducts of the paper, wood, and herbicide industries and are formed in the incineration of some chlorinated organic compounds (Schmidt, 1992). PCBs are a class of compounds that have approximately 113 congeners present in the environment. PCBs, which disrupt the hormone pathways (for example, male fertility) (Sager, 1983), were banned from further production in United States in 1976, under the Toxic Substances Control Act because these agents were used widely between 1930 and 1970 as an additive in products such as paints, plastics, rubber, adhesives, printing ink and insecticides (Peakall and Lincer, 1970). While 31% of total PCBs manufactured are currently estimated to be present in the global environment, only 4% of cumulative world production can be accounted for as degraded or incinerated. Many PCBs are still in use in older electrical equipment (e.g., transformers), in containment storage, or in dumps or landfills. Releases from these sources can result in continuing PCB pollution for years to come (Tanabe, 1988). Evidence also exists that pesticides such as alachlor, DDT, dicofol, methoxychlor, chlordane, and many others can disrupt the endocrine systems of fish and feral species.

Phytoestrogens: Certain Phytoestrogens, which are hormone-mimicking substances naturally present in plants, are suspected of interfering with the endocrine systems of grazing animals (Hughes, 1988). Specific compounds that have been identified as phytoestrogens include coumestrol, formononetin, daidzein, biochanin A, and genistein. In all, more than 300 species of plants in more than 16 families are known to contain estrogenic substances (Hughes, 1988). Some examples of plants that contain phytoestrogens include beets, soybeans, rye grass, wheat, alfalfa, clover, apples and cherries. These agents are responsible for the depression of fertility observed in sheep grazing on clover pastures, decreasing serum progesterone or pituitary LH. Plant sterols in paper pulp mill effluent also may be responsible for the masculinizing effect observed in fish downstream of pulp mills (Davis and Bartone, 1992). It should be noted that some phytoestrogens (e.g., naringenin) can be both estrogenic and antiestrogenic.

Endocrine-related Effects: Chemicals can affect normal endocrine function and certain disrupting chemicals can substantially reduce some animal populations. We know that there can be extreme differences in the susceptibility between species to these chemicals.

These differences are exploited specifically by chemists in the development of pesticides designed to disrupt insect endocrine systems through an array of compounds, which are collectively referred to as insect growth regulators. Thus, the endocrine systems of insects have been intentionally targeted for insecticidal activity. These chemicals include juvenile hormone mimics (methoprene), antijuvenile hormone analogs (precocene), chitin synthesis inhibitors (diflubenzuron), ecdysone analogs (tebufenozide), and molting disruptants (fenoxycarb). These insect growth regulators were developed to be not only efficient pesticides, but also to be highly specific to insects without risk to other nontarget animals, especially vertebrates. Although these compounds can be active against some insect species and not others, studies have documented the sensitivity of certain nontarget arthropods, especially crustaceans, to these compounds (Nimmo *et al.*, 1980; Touart and Rao, 1987). Besides the insect growth regulators, the well-known case of DDT and its effects on avian eggshell thinning has been linked to endocrine pathways. Evidence is accumulating that many chemicals released into the environment can disrupt normal endocrine function in a variety of fish and wildlife.

Some of the deleterious effects observed in aquatic and wild life that may be caused by endocrine-disrupting mechanisms, as summarized by Colborn *et al.* (1993), include the following:

(I) Abnormal thyroid function in birds and fish (Moccia *et al.*,1986)
(ii) Decreased fertility in birds, fish, shellfish, and mammals (Gibbs *et al.*, 1988)
(iii) Decreased hatching success in fish, birds, and reptiles (Kubiak *et al.*, 1989; Bishop *et al.*, 1991),
(iv) Demasculinization and feminization of fish, birds, reptiles, and mammals (Munkittrick *et al.*, 1991; Guillette *et al.*, 1994
(v) Defeminization and masculinizatio n of fish and gastropods (Davis and Bartone, 1992; Ellis and Pattisina, 1990),
(vi) Alteration of immune function in birds and mammals (Erdman, 1988; Martineau *et al.*, 1988).

Test Methods for Determination of Endocrine Disruption: A variety of test methods are available, but it is not known which one(s) is the best to determine the effects of endocrine-disrupting chemicals on fish and wildlife. While it is beyond the scope of this document to list and discuss various tests for each hormone and process, consider just one class of hormones-estrogens, for example. Several *in vitro* bioassay have been developed for assessing the estrogenicity of chemicals using human breast estrogen-sensitive MCF-7-cells (Gierthy *et al*, 1991; Soto *et al.*, 1992). The assays compare the cell yield after 6 days of culture in medium plus 10% charcoal-dextran stripped human serum with and without estradiol and chemicals suspected of being environmental estrogenic agents.

Many tests have been conducted to determine the endocrine action and potency of environmental chemicals by using developmental or physiologic effects as endpoints. Developmental effects are those that affect the developing organism and may result in irreversible changes. Physiological effects are those that occur any time after development and may be reversible. For example, Gellert and Wilson (1979) have demonstrated that the offspring of chlordecone (Kepone)-treated dams exhibit persistent vaginal estrus and anovulation. Eroschenko (1981) also reported that administration of Kepone to pregnant rats or mice during the main period of fetal organogenesis results in fetal toxicities and malformations in the offspring. As another example, a study by Gray *et al.* (1989) measured reproductive alterations in rats by age at vaginal opening, first estrus, and preputial separation in males being dosed with methoxychlor at 25, 50, 100, or 200 mg kg^{-1} day^{-1} from weaning through puberty, gestation to postnatal day 15. Methoxychlor accelerates the age at vaginal opening and first estrus. In the highest dosed group, females go from constant estrus into pseudopregnancy following mating, but do not implant. In males, methoxychlor treatment reduces growth, seminal vesicle weight, caudal epididymal weight, caudal sperm count, and pituitary weight.

Vitellogenin, whose relevance in fish has already been discussed, provides an example of a biomarker that may be determined very useful in assessing endocrine, especially estrogenic or other feminization, effects. A vitellogenin assay improved by developing a procedure to isolate rainbow trout hepatocytes, treat the cells with a suspected estrogen, and then measure the vitellogenin that is secreted into the culture medium. Jobling and Sumpter (1993) utilized this *in vitro* bioassay to evaluate the estrogenic activities of alkylphenol ethoxylates and their breakdown products.

The vitellogenin assay and the MCF-7 cell assay (Soto *et al.*, 1992) are methods that can screen for estrogenic activity. The results of these assays have actual implications for animals. For instance, nonylphenol has been shown to reduce testicular development in fish and also had a positive response in both assays. Likewise, octylphenol and its ethoxylates and benzyl butyl phthalate were estrogenic in the vitellogenin assay and both were found to reduce testicular size and sperm production in the offspring of female rats exposed to the substances via drinking water (Sharpe and Skakkebaek, 1993). Screening assays are not limited to breast cell cultures or hepatocytes. Scientists have developed an estrogen assay using the yeast *Saccharomyces cerevisae* to screen for estrogens, and this assay has been used to assess rivers for the presence of estrogenic compounds. The next challenging step will be to modify existing test methods or develop new ones to further evaluate the results of bioassay or other screening methods. For practical and cost reasons, tests will have to be developed in a tiered fashion. A consensus-building approach will be needed, and this area will be the subject of intense activity for some years to come. Furthermore, other endocrine disruption effects, in addition to estrogen or androgen mimics, will have to be evaluated as more information becomes available (Sharpe and Skakkebaek, 1993).

Development and use of tests targeting endocrine function could assist the risk assessor in the determination of whether a particular agent is an endocrine disruptor and of what toxicological significance. Of immediate need, however, is an array of test methods utilizing *in vitro*, whole animal, and field-level approaches for identifying, quantifying, and elucidating endocrine-related toxicological effects. A framework establishing the more useful of available methods and for linking or "tiering" these for a co-ordinated assessment of potential endocrine effects are also essential for prudent regulatory intervention.

Endocrine Disruptor Chemicals

Bisphenol A: Bisphenol A is a building block for making polycarbonate plastic used for structural parts, impact resistant glazing, street-light globes, household appliance parts, components of electrical/electronic devices, automotive applications, reusable bottles and food and drink containers, epoxy resins for coatings, electrical laminants, composites, adhesive, street-light globes, compact discs, reusable bottles, food and drink containers, and many other products. Cured epoxy resins are inert materials used as protective liners in metal cans to maintain the quality of canned foods and beverages and have achieved wide acceptance for use as protective coatings because of their exceptional combination of toughness, adhesion, formability and chemical resistance. Sixty-three percent of BPA is used in the manufacture of polycarbonate resins, 27% in epoxy resins and the remaining 10% in applications such as flame retardants and certain resin (Colborn *et al.*, 1993; colborn and clement, 1993). BPA exhibits toxic effects only at very high exposures. Realistically, such high exposures to consumers are not possible. Occupational exposures are well controlled.

There is no bisphenol A migration from polycarbonate plastics products under normal heating and storing of foods and beverages, using analytical limits of detection as low as 2 parts per billion. To achieve detectable levels of migration (2ppb limit of detection) of bisphenol A from polycarbonate plastics, polycarbonate has to be cut into strips and boiled in an alcohol solution for 30 minutes. These exaggerated use conditions do not represent normal consumer use of polycarbonate products. Some researchers (Colborn *et al.*, 1993; Colborn and Clement, 1992) have measured extremely

low levels of bisphenol A migration from epoxy can linings. This level is more than 475 times lower than the maximum acceptable dose for bisphenol A. Consequently, human exposure to bisphenol A from can coatings is minimal and poses no known health risk. There are no known health risks from low-level exposures to bisphenol A, toxic levels of BPA exposure result in weight loss in laboratory animals with other effects related to the weight loss as a consequence. A study looked for the effects on normal development of laboratory rats and mice exposed to BPA during pregnancy. This study concludes that even doses of BPA high enough to be toxic to the pregnant animals did not alter fetal development of the pups. Minute amounts of BPA have been detected in the environment; the levels are far below that can cause harm to wildlife or people. In cases where trace amounts of BPA have been detected in the environment, the levels have been far below those that have been shown to affect even the most sensitive species. The extensive safety data that exist for bisphenol A show that consumer products made with BPA are safe for their intended use.

DI-n-Butyl Phthalate: Di-n-butyl phthalate is an odorless and colorless oily liquid. The chemical formula is $C_{16}H_{22}O_4$, and the molecular weight is 278.35g mol^{-1} and vapor pressure is 1.0 x 10^{-5} mm of Hg at 25°C; it is use to help makes plastics soft and flexible. It is used in shower curtains, raincoats, food wraps, bowls, car interiors, vinyl fabrics, floor tiles and other products. Di-n-butyl phthalate appears to have a relatively low acute and chronic toxicity. Inhalation or oral exposure to di-n-butyl phthalate and the only effects noted in animals from inhalation exposure are minimal effects on the liver and a slight decrease in kidney weight. The Reference Dose (RfD) for di-n-butyl phthalate is 0.1mg Di-n-Butyl Phthalate kg^{-1} Food day^{-1}. Consumption of this dose or less, over a lifetime, would not likely result in the occurrence of chronic, noncancer effects. The RfD is not a direct estimator of risk but rather a reference point to gauge the potential effects. Exceedance of the RfD does not imply that an adverse health effect would necessarily occur. As the amount and frequency of exposures exceeding the RfD increase, the probability of adverse health effects also increases. Animal studies have reported developmental effects, such as reduced fetal weight, decreased number of viable litters, and birth defects in mice exposed orally (Gray *et al*., 1989). Reproductive effects, such as decreased spermatogenesis and testes weight, were also noted in oral animal studies (Colborn and Clement, 1993, Davis and Bartone, 1992). The largest source of exposure to di-n-butyl phthalate is from food; levels in food range from around 50 to 500ppb. Low levels (around 0.01 ppb) of di-n-butyl phthalate have been detected in ambient air. Higher levels (0.03 to 0.06ppb) have been found in urban air, and even higher levels can occur in the air of new cars and inside homes, especially when products containing di-n-butyl phthalate, such as vinyl floors, are installed. It is present in some drinking water supplies, usually at levels around 0.1 to 0.2ppb.

4-n-hexyioxyphenol: The chemical formula for 4-n-Hexyloxyphenol (HOP) is $C_{12}H_{18}O_2$, and its molecular weight is 194 g mol^{-1}, it is a white crystalline solid that is soluble in water. Tinnitus (ringing in the ears), dizziness, headache, nausea, vomiting, dyspnea, erosion of the gastric mucosa, edema of internal organs, cyanosis, convulsions, delirium, and collapse may result from the ingestion of a large amount of HOP in humans, it is also a skin irritant in humans. Chronic occupational exposure to hydroquinone dust has resulted in eye injuries, which varied from mild irritation and staining of conjunctivae and cornea, to changes in the thickness and curvature of the cornea, loss of corneal luster and impaired vision; prolonged exposure is required for the development of severe ocular effects. Reference Concentration (RfC) for HOP is 0.04mg $kg^{-1}d^{-1}$. Exceedances of the RfC dose not imply that an adverse health effect would necessarily occur. As the amount and frequency of exposures exceeding the RfC increase, the probability of adverse health effects also increases. A slight reduction in maternal body weight gain, decreased fetal weight, increased resorption rate and reduced fertility in males have been observed in rats orally exposed to HOP via gavage (experimentally placing the chemical in the stomach) or in the diet (Soto *et al*., 1991).

Occupational exposure to HOP may occur by inhalation or dermal contact HOP is released to the atmosphere from its production and use, such as during methyl methacrylate manufacture and in the production of coal-tar chemicals. It may be released in the effluent of photographic processes and from coal gasification condensate water. Individuals who develop black-and-white film may be exposed to HOP, as it is a common component of developing solutions, HOP has been detected in cigarette smoke and in diesel engine exhaust.

Rats chronically exposed via gavage (experimentally placing the chemical in the stomach) suffered from tremors and convulsions and death at the highest levels as well as toxic nephropathy and effects on the stomach and forestomach lesions were reported in mice. Rats exposed to HOP in their diet ate less, lost weight and developed aplastic anemia. Rats that consumed the chemical in their water gained weight more slowly; developed slight blood effects and dystrophic changes in the small intestines, liver, kidneys and myocardium; and had increased liver and kidney weights. Depressed weight gain has also been reported. In several animal studies, no significant health effects were noted. A slight reduction in maternal body weight gain, decreased fetal weight, increased resorption rate and reduced fertility in males have been observed in rats orally exposed to HOP via gavage or in the diet.

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