Special Communication

ENVIRONMENTAL ESTROGENS AND THEIR WILDLIFE AND HUMAN HEALTH EFFECTS: A REVIEW

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Estrogens are steroid hormones made primarily in the female ovaries and the male testes in humans and other animals. Known as the female hormones, estrogens are found in greater amounts in females than in males. These essential molecules influence growth, development and behavior (puberty), regulate reproductive cycles (menstruation, pregnancy), and affect many other body parts (bones, skin, cardiovascular system, immunity, the brain, etc.)

Estradiol is the most abundant and potent estrogen hormone. Estrone and estriol are other types of estrogen. Estrogen is commonly defined as "any of a family of steroid hormones that regulate and sustain female sexual development and reproductive function."[1] In the same vein, modern scientists define estrogens as materials that stimulate tissue growth by: 1) promoting cell proliferation (DNA synthesis and division) in female sex organs (breast, uterus); 2) promoting hypertrophy, or increasing a cell's size, such as occurs in breast and male muscle during puberty; and 3) initiating synthesis of specific proteins.[2] Under these guidelines, any natural steroid, plant compounds or synthetic chemicals that elicit these responses in laboratory tests are considered to be estrogenic.

The actions of estrogen were initially described by Stockard and Papanicolaou in 1917 with guinea pigs,[3] and by Long and Evans in 1922 using rats.[4] Both observed that preovulatory follicle swelling was followed by uterine lining growth and vaginal cell maturation. Later, Allen and Doisy isolated the responsible steroid, called it estrone, and described a test to detect this estrogenic activity in biological samples.[5] Since then, their test and/or similar ones have become the standard way to detect, identify and characterize natural and synthetic compounds with estrogenic activity.[6]

Environmental Estrogens

Environmental estrogens are endocrine disruptors, the synthetic chemicals and natural plant compounds that may affect the endocrine system. Although most are weaker than natural estrogen, many of these substances have been associated with developmental, reproductive and other health problems in wildlife and laboratory animals. Some experts suggest that these compounds may affect humans in similar ways. Many estrogenic contaminants are produced for specific purposes and are used in pesticides, plastics, electrical transformers and other products. Other substances are generated as byproducts during manufacturing, or are breakdown products of some other chemicals. Some, such as diethylstilbestrol (DES), are drugs, while others are natural plant compounds called phytoestrogens.

Natural hormones are short-lived, do not accumulate in tissue and are easily broken down by our bodies. Most of the natural estrogens stay in the blood stream for only a few minutes or at most a few hours.[7] After that, enzymes in the liver break the hormones into pieces, which are either flushed out as waste products or reused to build other molecules. The same holds true for phytoestrogens. They are easily broken down by our bodies, spend very little time in our system, and are not stored in fat or tissue when eaten. These plant compounds are either flushed out intact or after being broken apart, or are further changed and absorbed into the body where they can act like estrogens.

Although opinions vary on their benefits, the health effects of phytoestrogens are influenced by the age of the individual during exposure (for instance, fetus, child or adult), and the length and concentration of exposure. The estrogenic drugs such as ethynylestradiol found in birth control pills are more stable and remain in the body longer than natural estrogen, such as estradiol. However, they are not nearly as persistent as pesticides and other environmental estrogens. On the other hand, synthetic environmental estrogens: 1) are not easily or readily broken down; 2) are long lived, remaining intact in the environment and in living organisms for many years; and 3) can accumulate in the natural world and within the fat and tissues of animals and humans. Upon exposure, some of these estrogenic chemicals can be either flushed out of the animal or a portion can be absorbed into the body where they can collect (or bioaccumulate) in fat and muscle. Because most are lipophilic, they tend to congregate in fatty tissue and stay there for years. During stress, pregnancy or breastfeeding, these substances can be released from fat and redistributed or passed on to an offspring. The compounds can also be passed on through the food chain, collecting in top-of-the-chain predators such

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as humans, eagles and panthers. Recent studies have shown that samples of wildlife blood contain total DDT concentration of 1 ng per milliliter,[8] a level about 1000 times higher than concentrations of free estradiol in blood.[9] More than one study has also found that polar bears, seals and humans living away from industrialized areas in the relatively pristine Arctic region have significantly elevated levels of many different pesticides and industrial waste products.[10,11] This demonstrates the global distribution and impact that these chemicals have.

Some of the current theories on how environmental estrogens interact with the endocrine system state that ecoestrogens: 1) can bind to specific receptor sites inside the nucleus of cells and mimic or evoke a proper hormone response, block or inhibit a proper hormone response, both mimic and block hormones (polychlorobiphenenyls [PCBs] do both), elicit a weaker or stronger hormone response, or make a totally new response; 2) can bind to other receptors and create a novel reaction or interfere indirectly with normal hormonal action; and 3) can alter production and breakdown of hormone receptors and natural hormones which change hormonal blood concentrations and endocrine responses.

Many synthetic chemicals that also mimic estrogen are commercially manufactured for a specific purpose or produced as byproducts. Exposure to these substances occurs throughout our lives, from food, air, water, soil, household products and probably through breast milk and during development in our mother's womb. The human health risks that may be associated with these low-level yet constant exposures are still largely unknown and highly controversial.

Natural sex hormones travel in the blood stream searching out compatible receptor sites located in the nucleus of a cell. The hormones enter the cell, lock onto a specific receptor and turn on specific genes on the DNA. The genes tell the cell to make new proteins or other substances that can change cell functions (grow, divide or make more enzymes). Although natural steroid hormones generally function by binding to specific receptor sites, synthetic environmental estrogens affect the hormonal system in a number of different ways. They can interact directly with hormone receptors, where they may have little or a very different effect than natural estrogens. The chemicals can even block normal action. The most well studied effect came from compounds that simply bind to a hormone receptor and mimic or block normal hormone responses. It now appears that some environmental hormones produce their effects via elaborate signaling pathways and other complex modes of action that are independent of binding hormone receptors.

Foreign compounds, although different in shape from natural hormones, can travel in the bloodstream, enter a cell, bind with a receptor and trigger gene expression. Once bound with the receptor, the mimicker can produce a normal hormone response, cause an abnormal response, or have no response as it blocks the receptor site and interferes with normal hormone binding.

Not all endocrine disruptors alter hormonal action by binding to hormone receptors. Some relay molecular messages through a complex array of cellular proteins that indirectly turn genes on and alter cell growth and division. For instance, beta-hexachlorocyclohexane (beta-HCH) produces estrogen-like responses (cell division and growth) at levels found in human breast cancer tissue.[12] The compound which does not bind the estrogen receptor[12,13] may promote DNA transcription, and thus produce estrogenic responses, by passing signals on.[12] In another study, p,p'-DDE at or below levels found in human breast fat tissue bypassed the estrogen receptor and stimulated a complex mixture of cell-signaling proteins (growth factor receptors) and processes that eventually led to cell division.[14] But p,p'-DDE can also bind the androgen receptor and inhibit androgen binding.[15] The multiple signaling pathways initiated by beta-HCH and p,p'-DDE led to cell division by influencing the estrogen receptor without binding to it. Several factors confound how environmental estrogens affect the endocrine system.

1) Natural estrogen production varies with gender, age and reproductive cycles. Thus, environmental estrogen may have different influences (mimicking, blocking or cancelling out estrogen's effect) depending on the present estrogen levels.
2) Natural hormones are more potent than any of the known synthetic environmental estrogen (except drugs such as DES and birth control pills).
3) Some phytoestrogens, in very high amounts, cause infertility in farm animals and wildlife. Others seem to protect against breast cancer and reproductive cancer in humans.
4) A combinations of certain synthetic compounds may have a synergistic effect.[16]
5) Synthetic estrogens may also antagonize each other's effect, i.e., a weakly antiestrogen compound may cancel out a weakly estrogenic compound and produce no effect.[17]

Some believe these environmental compounds can affect human health, development and reproduction in similar ways, although this has not yet been scientifically proven. The idea that man-made chemicals have adverse health effects, including endocrine disruption, is not entirely new.

Effect of Environmental Estrogens on Wildlife

A study in 1938 found that certain synthetic chemicals could mimic estrogen,[18] and more than 30 years ago, Rachel Carson's *Silent Spring* described how some synthetic chemicals were collecting in and contaminating water, soil, wildlife and even humans. These chemicals, she warned, were causing severe health problem (eggshell thinning, cancer, die-offs) in wildlife, especially in species at the top of the food chain that eat other contaminated animals and accumulate the most chemicals. At high doses, some of these chemicals can affect an animal's endocrine system, especially during critical development stages. For instance, one study showed that fish living near municipal

sewage outlets in England had both male and female sex characteristics and their liver produced vitellogenin, a female egg-yolk protein not normally found in males.[19] Several different chemicals, especially the alkylphenols, the breakdown products of chemicals found in detergents and plastics, are suspected of causing the feminizing effects. Alligators living in Florida's Lake Apopka were exposed to the estrogenic pollutants dicofol, DDT, DDE and chloro-DDT, when a chemical plant had an extensive spill in 1980. Ten years later, researchers trying to find out why the alligator population was dropping in the lake found higher than normal mortality among eggs and newborn alligators. Researchers from the University of Florida in Gainesville (US), surveying the hatching rates of alligators from various lakes, found that between 80% and 95% of alligator eggs from Lake Apopka failed to hatch, compared to 20% to 30% for other lakes. They also found that adolescent females had severe ovarian abnormalities and had blood estrogen levels two times higher than normal. The male juvenile alligators were feminized, that is, they had penises one-half to one-third the normal size, had abnormal testes and higher estrogen levels and lower testosterone level in their blood than normal males of the same age. The researchers concluded that chemicals from the spill not only killed developing eggs outright, but also altered the embryo's endocrine system, which severely limited the alligators' ability to reproduce.[20,21] The lake contained up to 15% DDT or DDE, the even more toxic product that forms as DDT breaks down.

Studies of mammals, reptiles, birds and fish, as well as laboratory studies using rodents, primates and cultured cells have linked exposure of a developing embryo to environmental contaminants with many permanent health effects in the adult. The effects include abnormal blood hormone levels, reduced fertility, altered sexual behavior, modified immune system, masculinization of female, feminization of males (reduced testes and penis size), undescended testicles, cancers of the female and male reproductive tract, malformed fallopian tubes, uterus and cervix, and altered bone density and structure.

Some wildlife biologists believe that endocrine-disruption chemicals are placing many wildlife populations at risk. Exposure to environmental contaminants has been linked to documented problems in wildlife that include thyroid dysfunction in birds and fish; decreased fertility in birds, fish, shellfish, and mammals; decreased hatching and gross birth deformities in birds, fish and turtles; male fish, birds and mammals that are feminized; female fish, birds and mammals that are masculinized; and compromised immune systems in birds and mammals. Many of these effects are manifested in species living in or near the Great Lakes (USA), which contain many synthetic chemicals, including byproducts of chemical production such as PCBs and pesticides. PCBs were used for a variety of purposes, including electrical transformation and capacitors. Although their use is now severely restricted, PCBs are still found in other electrical stations and other machinery.

The 209 different PCBs differ greatly in their estrogenic potency. Most PCBs do not readily degrade, and they accumulate in marine animals, birds and mammals.[22] Pesticides like DDT, DDE, dieldrin, dicofol, kepone, mirex, etc., have been shown in tissue and biological assay to be weakly-to-moderately estrogenic.[23,24] Over the past few years since the problem became evident, a number of different assay techniques have been developed. For egg-laying vertebrates, a technique was developed to test for the production of vitelligenin by liver cells in response to estrogen or estrogenic substance.[25] For mammals, a different technique had to be developed, since they do not produce vitelligenin. A classic technique to test for estrogenicity is the proliferation and cornification of mouse vaginal epithelium. Another technique for testing a substance for estrogen is the induction of lactoferrin in the mouse uterus. Lactoferrin was found to be a sensitive marker for estrogen in the mouse uterus.[26] Soto et al. believe they have come up with an easier and more inexpensive way of testing estrogenicity.[23] They introduced a proliferative assay technique (E-SCREEN) that uses human breast cancer estrogen-receptive MCF-7 cells. These cells need estrogen to proliferate into tumors. Their assay technique produced 12 different insecticides that tested positive for estrogen activity,[23] the most notable being DDT, dieldrin, methoxychlor and kepone. If the chemicals tested test positive in this assay, then they are likely to cause proliferation in estrogen-sensitive cells in an animal's body (i.e., endometrium, breast tissue), and this proliferation could lead to cancer formation.[27] Fry and Toone found that when they injected fertilized sea bird eggs with DDT and its metabolites, the male chicks produced had varying degrees of intersexuality depending on dosage. The female chicks had partial-to-fully developed right oviducts, instead of just left function structure.

The discovery of a true hermaphroditic beluga whale is believed to be a result of the high organochlorine pollution in the St. Lawrence River.[28,29] Because the effects of exposure are often delayed, they may not be fully expressed until the offspring of the exposed adult reaches maturity or even middle age. Thus, the reproductive future for some species may be grim even if adults continue to breed and juveniles seem healthy.

Cryptorchidism of male Florida panthers is now suspected to be caused by the eating of high pesticide-laden prey. Cryptorchidism reduces the fertility of the animal.[30] Additional evidence by Gellert showed that when neonate rats were injected daily until 6 months of age with the pesticide kepone, this caused reproductive effects on the neonate animal. The effects of the pesticide were persistent vaginal estrus, anovulation and precocious vaginal opening. A study by Nelson showed that dieldrin and mirex stimulates swimming in sea urchin, and lindane inhibits sperm motility.[31] A different study showed that mirex caused testicular cancer in humans.[31]

Effect of Environmental Estrogens in Humans

More American women have died of breast cancer in the past two decades than all the Americans killed in the two world wars, the Korean War and Vietnam War combined.[32] About 182,000 new cases of breast cancer arise each year among US women.[33] Furthermore, since 1940, the incidence of breast cancer has been creeping up by approximately 1% each year. This relentless increase cannot be explained by an aging population or by better detection such as mammography screening.[33] Since 1940, a woman's chance of getting breast cancer has doubled.[34] Everyone now accepts that breast cancer has environmental and "lifestyle" causes. Two basic facts make this conclusion inescapable. First, breast cancer incidence is five times as high in some countries as in others. Second, when a woman migrates from a country with low incidence of breast cancer to a country with high incidence, their daughters acquire the breast cancer risk prevailing in the high-incidence country.[35] Clearly, something in the environment (air, water, soil, food, or electromagnetic spectrum, e.g., x-rays) is at work here. In the past five years, researchers have begun asking whether pesticides and plastics and other chlorinated chemicals can interfere with both male and female sex hormones in wildlife and humans.[36] They have posited that if 30% of breast cancer is known to be caused by naturally occurring female sex hormones, then is there not a reasonable likelihood that some of these chlorinated chemicals contribute to the rising incidence of breast cancer? It seems a reasonable enough question. Researchers Davis et al. of Cornell University formally proposed a hypothesis suggesting ways in which environmental estrogens might cause breast cancer.[37]

The evidence that environmental estrogens affect wildlife is strong evidence of similar risks to human beings. Estrogen mimics are extremely potent in part because unlike most natural estrogens, they cross the placental barrier, exposing the fetus to greater than normal levels of hormones. If a fetus or neonate is exposed to estrogenic chemicals early in life, studies have shown that a number of reproductive abnormalities can develop. Sons born to mothers who ate food contaminated with polyhalogenated compounds have undersized penises and testicular malformations.[38] Daughters of mothers who took the synthetic estrogen DES during pregnancy to prevent miscarriage have higher rates of reproductive problems, reproductive cancer and malformations of the cervix, oviducts, uterus and vagina, as well as anatomical masculinization.[39,40] Sons may also face higher rates of malformed or small penises, hypospadias, undescended testicles, abnormal sperm, and testicular cancer.[40-42] However, a recent study found no evidence of reduced fertility in DES sons.[43]

DES is not only a potent estrogen similar in strength to the natural estrogen estradiol, but it also has the unique ability to concentrate in target tissue, such as the reproductive tracts of birds, reptiles and other animals, during development and can cause abnormalities.

Considerable controversy surrounds a study that found that sperm counts in men were falling worldwide, that rates of testicular cancer were increasing, and that environmental estrogens may be responsible for these trends.[42] Other studies do not support all of these findings and state that lower fertility and higher cancer rates may only occur in certain human populations.[44] Aside from the drug DES, environmental estrogens have never been proven to cause human health problems, so we can only speculate on possible human health effects documented from animal studies. For women, breast and reproductive organ tissue cancers, fibrocystic disease of the breast, polycystic ovarian syndrome, endometriosis, uterine fibroids and pelvic inflammatory diseases may be influenced by developmental or chronic lifetime exposure to estrogen mimics. For men, poor semen quality (low sperm counts, low ejaculate volume, high number of abnormal sperm, low number of motile sperm), testicular cancer, malformed reproductive tissue (undescended testes, small penis size), prostate disease and other recognized abnormalities of male reproductive tissue may occur. In men, some studies indicate that the estrogenic compounds affect the development of Sertoli cells in the testicles. These cells secrete masculinizing hormones that regulate sperm production, the descent of the testicles, and the development of urethra.

Researchers in Europe and North America are studying a possible link between exposure to estrogen disruptors during key points in the development of reproductive organs and systems and increasing incidence in some industrialized countries of prostate and testicular cancer, as well as lower sperm counts and sperm value, undescended testes in newborn males, and abnormalities of the urinary tract. Already, undescended testes are a known risk factor for testicular cancer. They also suspect that exposure to pesticides and endocrine-disrupting chemicals may be related to health problems in the US, including a 400% increase in ectopic pregnancies between 1970 and 1978, and an increase in female breast cancer between 1969 and 1986.

Some studies have also shown that a woman with breast cancer has higher blood levels of DDE than a cancer-free woman. DDT and its metabolites have been shown in some studies to cause breast cancer in humans and mice with high chemical loads.[45,46] Researchers are looking for possible mechanisms by which artificial estrogen interacts with breast cancer susceptibility genes, thereby fostering cancer. If the connection between environmental estrogens and breast cancer could be proven, it might be possible to lessen the risk of this disease by reducing exposure to the chemicals.

A study by Bradlow et al.[49] found that organochlorine pesticides increase the breakdown of estrogen into 16 u-hydroxyestrone, which cause uncontrolled proliferation. This cell proliferation is one of the leading causes of pesticide-induced breast and endometrial cancer. The pesticide methoxychlor causes decreased sperm motility, body weight and a slight decrease in sperm count.[47] The

fungicide benomyl produces premature release of germ cells, testicular swelling and occlusion of efferent ductules. Seminiferous tubular atrophy was also observed.[48] Since the problem of the estrogen-active pesticides became apparent in the environment, scientists are advocating that all pesticides and their metabolites should be tested not only for carcinogenicity but also hormonal activity.

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