Recently, a number of ecologists, epidemiologists, endocrinologists, and toxicologists have called attention to the potential hazardous effects that estrogenlike and antiandrogenic chemicals and certain other environmental chemicals may have on human health and ecological well-being. A hypothesis has been proposed that certain chemicals may disrupt the endocrine system. These chemicals have been called "endocrine disruptors" because they are thought to mimic natural hormones, inhibit the action of hormones, or alter the normal regulatory function of the immune, nervous, and endocrine systems. Possible human health endpoints affected by these agents include breast cancer and endometriosis in women, testicular and prostate cancers in men, abnormal sexual development, reduced male fertility, alteration in pituitary and thyroid gland functions, immune suppression, and neurobehavioral effects.

In addition to potential human health effects, reports have accumulated that many chemicals released into the environment can disrupt normal endocrine function in a variety of aquatic life and wildlife. Some of the deleterious effects observed in animals have been attributed to some persistent organic chemicals such as polychlorinated biphenyls, DDT (dichlorodiphenyltrichloroethane), dioxin, and some pesticides. Adverse effects include abnormal thyroid function and development in fish and birds; decreased fertility in shellfish, fish, birds, and mammals; decreased hatching success in fish, birds, and reptiles; demasculinization and feminization of fish, birds, reptiles, and mammals; defeminization and masculinization of gastropods, fish, and birds; decreased offspring survival; and alteration of immune and behavioral function in birds and mammals. It has been proposed that the above adverse effects may be due to an endocrine disrupting mechanism.

**Nature of Hormones**

Hormones are natural, secretory products of endocrine glands (ductless glands that discharge directly into the bloodstream). 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 bodily functions.
Role of the Endocrine System
The endocrine system is one of at least three important integrating and regulatory systems in humans and other animals. 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; and 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).

Human Health Effects
Female Reproductive System
A variety of chemicals have been shown to disrupt female reproductive function throughout the lifespan in laboratory animals and humans (e.g., diethylstilbestrol). These effects include the disruption of normal sexual differentiation, ovarian function (i.e., follicular growth, ovulation, corpus luteum formation and maintenance), fertilization, implantation, and pregnancy.

Male Reproductive System
Convincing evidence exists in rodents that exposure to chemicals that have estrogenic activity, reduce androgen levels, or otherwise interfere with the action of androgen during development can cause male reproductive system abnormalities that include reduced sperm production capability and reproductive tract abnormalities. Results obtained from observation of men exposed to DES in utero demonstrate a limited potential of exogenous estrogens to disrupt the reproductive system during development in human males as compared with that observed in rodents. Further intense research on the population exposed to DES might allow stratification of effects by timing and level of exposure. Apparently, no increased incidence of reproductive system cancer has been found in those men.

Controversy persists as to the allegation that human sperm production has decreased over the past 50 years. However, the firm data indicating an increase in human testicular cancer, as well as apparent occurrence of other plausibly related effects, support the concept that an adverse influence has occurred or still exists. Whether these effects in humans can
be attributed to an endocrine disruption by environmental chemicals is unknown at present, and research into the cause(s) is needed. It is possible that the mechanism by which estrogenic chemicals impair development of the male reproductive system is via antiandrogenic properties rather than or in addition to activity related to estrogen receptor activation.

Testing for endocrine-disrupting potential of environmental chemicals should include the ability to detect antiandrogenic activity in addition to estrogenic activity. Testing also should be able to detect alteration in androgen receptor and Ah receptor function as reflected in genome expression.

**Hypothalamus and Pituitary**

There are a number of ways that environmental agents may alter neuroendocrine function both during development and in the sexually mature organism. Exposure during development may be of particular concern because many of the feedback functions of the endocrine system are not operational during this period, permanent changes in endocrine function may be induced at levels of exposure to a toxicant that may have no effect in the adult animal, and compounds that may be considered antiestrogenic in the adult (i.e., tamoxifen, dioxin) may act as estrogens in the developing organism. Similarly, exposure to such agents in the adult can modify the feedback of endogenous hormones as well as behavior (i.e., libido, appetite, aggression) of the individual. Because of the complex role that the central nervous system plays in regulating endocrine function, cells within the brain are a potential target for environmental chemicals that have no impact on steroid hormones directly but yet will lead to a disruption of endocrine function. There is a substantial need to better characterize the role of the brain and pituitary when evaluating suspected reproductive toxicants in both the male and female.

**Thyroid**

Numerous environmental agents have been found to alter thyroid hormone levels (e.g., urea derivatives, polyhalogenated biphenyls, and chlorinated dibenzo-p-dioxins). Thyroid hormones are critical to normal growth and development; thus, developmental exposures may have lasting adverse effects.
Ecological Effects
A number of laboratory and field investigations have been reported that provide information from which the potential effects of certain chemicals on the normal endocrine function of invertebrates, fish, reptiles, birds, and mammals can be evaluated. Based on these studies, it has been suggested in the literature that both synthetic and naturally occurring compounds may have the potential to disrupt reproductive and developmental events associated with hormonally mediated processes. In some cases, compounds have been deliberately synthesized for their potential to disrupt endocrine systems. For example, several classes of insecticides have been developed to selectively disrupt the endocrine system of specific insect species without creating substantial risk to nontarget vertebrates due to direct toxic effects, although adverse responses in nontarget arthropods, especially crustaceans, have been observed.

A series of field and laboratory investigations with marine invertebrates suggest that tributyltin compounds, which are used as antifouling paints on ships, can have significant hormonal effects on some snail species at sublethal exposure concentrations. Through controlled dose-response studies, it appears that these compounds can induce irreversible induction of male sex characteristics on females (imposex), which can lead to sterility and reduced reproductive performance.

A wide variety of compounds and environmental settings also have been associated with potential reproductive and developmental anomalies in fish. For example, hermaphroditic fish have been observed in rivers below sewage treatment plants, and masculinization, altered sexual development, and decreased fertility have been noted for some fish species near pulp and paper plant discharges. It is unclear from these studies, however, as to the extent to which these observations are associated with significant changes in population dynamics. In addition, it is generally unclear as to the primary causes of these perturbations. However, correlative data, supported in some cases by controlled laboratory studies, suggest that alkyl phenol ethoxylates and their degradation products, chlorinated dibenzodioxins and difurans, and polychlorinated biphenyls (PCBs), among other compounds, could be contributing causative agents.
Perhaps the most fully documented example of putative ecological effects caused by a disruption of endocrine function has been reported for alligators in Lake Apopka, Florida. Through a series of detailed field and laboratory investigations, it appears very likely that a mixture of dicofol, dichlorodiphenyltrichloroethylene (DDT), and dichlorodiphenyldichloroethylene (DDE) associated with a pesticide spill in 1980 is responsible for a variety of developmental effects that indicate a demasculinization of male alligators and "super-feminization" of females. In addition, the effects of the spill also have been reported to include detrimental effects on hatching success and population levels.

Instances of potential effects of chemicals on the endocrinology of warm-blooded wildlife also have been reported. For example, a variety of organochlorine insecticides have been implicated in eliciting feminization of male gull embryos and has led to the suggestion that these effects may contribute to locally observed population declines and skewed sex ratios in Western gulls in California and Herring gulls in the Great Lakes. Although numerous controlled laboratory studies have been undertaken that demonstrate a variety of compounds can elicit hormonally mediated effects on reproduction and development in rodents, the establishment of credible cause-and-effect relationships in wild mammalian populations has not been reported in the scientific literature to date, although the extreme sensitivity of mink, seals, and related species to adverse reproductive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and PCBs is well established.

**Endocrine/hormone Disruptors**

An environmental endocrine or hormone disruptor may be defined as an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body 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. Under some circumstances, they 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.

**What are Endocrine Disruptors?**

Endocrine disruptors are chemicals which interfere with endocrine system function. An endocrine system is found in nearly all animals, including mammals, non mammalian vertebrates (like fish, amphibians, reptiles, and birds), and invertebrates (like snails, lobsters, insects, and other species). The endocrine system consists of glands and the hormones they produce that guide the development, growth, reproduction, and behavior of human beings and animals. Some of the endocrine glands are the pituitary, thyroid, and adrenal glands, the female ovaries and male testes. Hormones are biochemicals, produced by endocrine glands, that travel through the bloodstream and cause responses in other parts of the body. The Endocrine Disruptor Screening Program will focus on the estrogen, androgen and thyroid hormones.

Estrogens are a group of chemically similar hormones responsible for female sexual development; estrogen is produced mainly by the ovaries, but also by the adrenal glands. Androgens are substances, usually hormones, responsible for male sex characteristics. Testosterone, the sex hormone produced by the testicles, is an androgen. The thyroid gland secretes two main hormones, thyroxine and triiodothyronine, into the bloodstream. These hormones stimulate all the cells in the body.
Hormones can produce both positive and negative effects. For example, some types of breast cancer are exacerbated by estrogen, but studies also indicate that estrogen has a protective effect in combating heart disease and osteoporosis related fractures in older women.

Disruption of this complex system can occur in various ways. For example, some chemicals may mimic a natural hormone, "fooling" the body into over responding to the stimulus or responding at inappropriate times. Other chemicals may block the effects of a hormone in parts of the body normally sensitive to it. Still others may directly stimulate or inhibit the endocrine system, causing overproduction or underproduction of hormones. Certain drugs are used to intentionally cause some of these effects, such as birth control pills.

An example of the devastating consequences of exposure of developing animals, including humans, to endocrine disruptors is the case of the potent drug diethylstilbestrol (DES), a synthetic estrogen. Medical doctors prescribed DES to as many as five million pregnant women to block spontaneous abortion prior to DES being banned in the early 1970's. DES was prescribed in the mistaken belief that it would prevent miscarriage and promote fetal growth. It was discovered after the children went through puberty that DES affected the development of the reproductive system and caused vaginal cancer. Since then, Congress has improved how drugs and other chemicals are evaluated and regulated B requiring that an endocrine disruptor screening program be established is a recent and significant step.

Although regulatory policy concerning endocrine disruptors is still evolving, EPA has already taken regulatory action on some chemicals of concern through the pesticide and toxic substances programs. Organochlorine compounds, such as polychlorinated biphenyls (PCB=s) and chlorinated pesticides, have long been problematic in the environment for a number of reasons, and many of them (like DDT) have endocrine activity.

The term organochlorine refers to chemical compounds that have a chlorinated hydrocarbon structure, that is, they are formed from atoms of hydrogen, carbon, and chlorine. Although their effect may be much weaker than the body=s natural hormones (like estrogens, androgens, and thyroid hormones), they are nonetheless suspected of disrupting the endocrine
system, resulting in harmful effects like reproductive and developmental defects and certain cancers. EPA has banned PCB=s, dieldrin, DDT, chlordane, aldrin, kepone, mirex, endrin, and toxaphene. Organochlorine pesticides still registered for use in the United States include endosulfan, lindane, methoxychlor, dicofol, dienochlor, and heptachlor. However, their use is very restricted and most are scheduled for priority pesticide registration review. They will likely be among the first compounds to be screened in the Endocrine Disruptor Screening Program.

Questions about Endocrine Disruptors (From the EPA)

* What is the endocrine system?
The endocrine system is composed of ductless glands that secret hormones into the blood stream to act at distant sites. Together with the nervous system, the endocrine system is responsible for the integration of many different processes which allow complicated organisms to function as a unit (maintain homeostasis).

* What are hormones?
Hormones can be proteins, polypeptides, amino acids, or steroids. The most well known hormones are the sex steroids estrogen, produced in the ovaries, and testosterone, produced in the testes. Estrogen and testosterone are also produced in the adrenal glands of both sexes. Other hormones include thyroxin, produced in the thyroid, and insulin, produced in the pancreas. The pituitary and hypothalamus in the brain release a variety of hormones that affect other organs, including the sex glands.

* How do hormones work?
From the blood, hormones interact with cells by binding to special proteins called receptors. The binding is specific, like a key in a lock. When enough binding sites are occupied, then a message is passed on to the target cell nucleus unmasking genetic information which results in physiological reactions ultimately responsible for stimulating or regulating proper metabolism, development, growth, reproduction, and behavior. For example, in women estrogen works in this way to control the menstrual cycle, and in men testosterone controls sperm production. Hormones are released into the blood in very small amounts. Their levels are controlled by the rate of release, and the rate of degradation, usually by the liver or
kidneys. Timing of hormone release is often critical for normal function. This is especially true during fetal development. Precise hormone control is important, as too much or too little at the wrong time can result in dysfunction of one or several body systems.

* How do scientists study hormones?*
In laboratory studies, glands can be removed or hormones can be administered to study the effect on animals, such as rats or mice. Human diseases or inherited disorders, which involve hormone imbalance, are studied to better understand the resulting dysfunction and to develop treatments. For example, it is known that men produce small amounts of estrogen and women produce small amounts of testosterone. Too much estrogen in a man results in female characteristics such as enlarged breasts. Too much testosterone in a woman results in male characteristics such as facial hair. Hormone imbalance can be life threatening, as with insulin and diabetes. Estrogen imbalance has been implicated in certain forms of breast cancer.

* What is an endocrine disrupter?*
There are chemical substances, sometimes called environmental estrogens, both from natural sources and man made, that if present in the body at the right concentration and at the right time, can adversely effect hormone balance or disrupt normal function in the organs that hormones regulate (modulate?). By EPAÆs working definition, endocrine disruptors "interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis (normal cell metabolism), reproduction, development, and/or behavior." Many endocrine disruptors are thought to mimic hormones. They have chemical properties similar to hormones that allows binding to hormone specific receptors on the cells of target organs. However, endocrine disruptor chemistry varies greatly, as does potency the effectiveness in binding and "turning on" the response. Most endocrine disruptors have very low potency as their chemistry is significantly different from the hormones they mimic. Lower potency means that a greater amount of endocrine disruptor is required to elicit the same response of the hormone they mimic. In addition to potency, the potential for a hormone like effect depends on dose. For all known endocrine disruptors there is some dose, below which there will be no effect. At doses slightly above this threshold some endocrine disruptors elicit a beneficial effect,
whereas at higher doses the effect is adverse (harmful).

* What chemicals cause endocrine disruption?*

Drugs have been specifically designed to treat hormone imbalance in humans. Diethylstilbesterol (DES), a drug with strong estrogenic properties administered to pregnant women until 1971 to prevent miscarriages, is a tragic example. Female children of mothers who took DES during pregnancy have a higher incidence of certain forms of ovarian and vaginal cancer. However, there are many drugs that mimic or otherwise affect hormone balance which are important to modern medicine. Other man made chemicals, with unintentional hormone like activity include: pesticides such as DDT, vinclozolin, endosulfan, toxaphene, dieldrin, and DBCP, and industrial chemicals and byproducts such as polychlorinated biphenyls (PCBs), dioxins, and phenols. Some of these phenols are breakdown products of surfactants, found in soaps and detergents. Also implicated are heavy metals, plastics, cosmetics, textiles, paints, lubricants. Sewage treatment effluent may contain a variety of natural and man made endocrine disruptors, including natural hormones from animal and human waste.

Currently, there are no standard tests to determine if a chemical is an endocrine disruptor. However, both the Clean Water Act and the Food Quality Protection Act require the EPA to develop test methods by 1999. As many endocrine disruptors are thought to affect sex hormone function, and therefore reproduction, the findings in multigeneration animal studies, currently required for pesticide registration by EPA, can provide strong evidence of the potential for endocrine disruption.

* What natural chemicals have endocrine activity?*

There are natural chemicals in plants that have hormone like activity. These chemicals, mostly phytoestrogens, are found in high levels in broccoli, cauliflower, soybeans, carrots, oats, rice, onions, legumes, apples, potatoes, beer, and coffee. Most phytoestrogens have weak activity (low potency) and people who consume diets rich in these substances may have a reduced risk of developing some hormone related diseases. However, the actual health risk or benefit of a diet rich in plant hormones is largely unknown. Some researchers argue that dietary consumption of plant hormones dwarfs the potential exposure from man made sources.
Isoflavones (in legumes, particularly soybeans):
  - Genistein
  - Daidzein
  - Biochanin A
  - Formononetin

Lignans, or resorcylic acid lactones (in whole grain cereal food, fibers, flax seeds, and probably berries and nuts):
  - Matairesinol
  - Secoisolariciresinol
  - Enterolactone
  - Enterodiol

Coumestans, coumestrol (in forages, clover, and legumes)
  - Equol is an estrogenic substance that results when intestinal bacteria break down either formononetin or daidzein.

* What is the relationship between hormones and cancer?*
Most scientists do not believe that hormones cause or initiate cancer, but some hormones may promote cancer growth. This promotion may result in cancer that appears at a younger age than expected, or in a cancer that grows at a faster rate. These findings suggest that chemicals that act like hormones may also promote cancers. In women, estrogen is thought to play a role in the promotion of some forms of breast cancer. Based on a single epidemiological study, the presence of DDE, a metabolite of DDT, has been associated with increased risk of breast cancer. However, more recent studies provide strong evidence that there is no relationship between DDE exposure and breast cancer. Other studies suggest that specific phytoestrogens and certain PCBs and dioxins can block estrogen from promoting some forms of breast cancer.

* What evidence is there that environmental contaminants are causing endocrine disruption in humans or wildlife?*
Recent studies of wildlife, including alligators, birds, and fish, have investigated the relationship between chemical exposure and reproductive problems. Many of these studies have shown that exposure to high doses can result in malformed reproductive organs, consistent with sex hormone imbalance at a critical stage of fetal development. Studies where very high doses of dioxin were fed directly to pregnant rats show effects on sexual development, sperm production, and sexual behavior in male pups. Directly feeding very high doses of DDT to rats has also shown adverse...
effects on sexual development. The dramatic results of these high dose studies has lead to speculation by toxicologists that the risk to reproductive success, associated with exposure to much lower levels of some chemicals in the environment, may be unacceptable.

In humans, a recent epidemiology study suggesting that sperm counts have declined by almost 50% over the past 50 years, and that this decline is associated with increased exposure to synthetic chemicals, has made the headlines. Other epidemiologists who have examined the same data do not reach this conclusion. Direct evidence of chemical effects on male fertility has been demonstrated in the study of workers involved in the manufacture of the older pesticides dibromochloropropane and leptophos (neither currently registered in the US). Again, such direct evidence is the result of exposure to very high doses.

* Should I be worried about endocrine disrupting chemicals?  
The number of chemicals that have been implicated as potential endocrine disruptors is substantial. There are many opportunities for exposure to these chemicals, both singly and as mixtures, albeit usually at very low levels. There are a variety of adverse health effects related to endocrine system dysfunction. This is not surprising, as the basic function of the endocrine system, in addition to controlling sex characteristics and reproductive functions and responding to perceived hostility (adrenaline release), is to maintain homeostasis (even keel) among the diverse functions of the body. Of specific concern is that a single relatively small dose at the right time during pregnancy can effect the fetus in ways that will not show until adulthood, and may impact the next generation due to decreased reproductive success. Also of concern is that these chemicals have been implicated in the increased incidence of certain cancers, including breast cancer.

So what is the risk? What is known with an acceptable degree of certainty is that high doses of some of these chemicals can cause adverse health effects in humans and animals. As the adverse health effects observed in these studies are biologically plausible there is reason for concern. Significant research and biological plausibility also support that responses to endocrine disruptors are dose/potency related: there is a no effect threshold. In addition, low doses of some substances may result in beneficial effects. Current information on likely levels of exposure does not
indicate a significant risk of adverse health effects to humans or wildlife. However, EPA has identified "Hot Spots" where adverse effects to wildlife have been observed. Human epidemiology studies which have examined the relationship between the manufacture and use of endocrine disruptors and health effects are far from conclusive. However, as the findings in these studies do not allow the identification and contribution of all risk factors, endocrine disruptors cannot be ruled out. Consequently, EPA and other federal and state agencies will continue to provide substantial funding for research to better understand the risks posed by endocrine disruptors.

**Endocrine Disruption - Sources of Evidence**

1. **Wildlife Studies:**

   A. Wildlife embryos exposed to estrogenic contaminants show lifetime health effects such as abnormal genitals, increased mortality and lower fertility.

   B. More than 600 common carp were collected and analyzed from 25 streams in 13 States. The streams selected drain areas with a wide range of land uses and different degrees of contamination. Results of the study indicate significant differences in sex hormones and vitellogenin, an estrogen-controlled protein necessary for egg development in fish and birds. Correlations between contaminants and the levels of sex hormones in carp indicate that some of the site-to-site differences were associated with certain contaminants (pesticides, phenols, organochlorines).

   C. Juvenile rainbow trout were caged both upstream and downstream from sewage treatment works. Blood plasma vitellogenin was analyzed by ELISA, which indicated that vitellogenin production was induced in the males and juveniles of the down-stream fish.

   D. 70 Largemouth Bass were captured from the Escambia and Blackwater rivers. In the Escambia River, fish were collected immediately downstream of the Crist Electric Generating Plant (a coal-fired electric utility) and the Monsanto Company (a nylon fiber and chemical intermediates manufacturing plant). Blood samples were taken.
Laboratory results indicated that male fish from the contaminated site had significantly lower levels of circulating testosterone than the reference-site fish.

E. In 1989 a female Florida Panther was found dead in the Everglades National Park, with no obvious cause of death. Later tests revealed her liver contained high levels of mercury (110 ppm), and her tissue also contained high levels of PCBs (polychlorinated biphenels) and pesticide residues.

F. The incidence of testicular cancer increased 59% from 1964-1996. The relative increase was greatest in men aged 15-29.

2. Human Clinical and Health Trend Data:
3. A. Data gathered from health-care clinics over the past 40 years showed a statistically significant trend towards lower sperm quality (lower volume, numbers and motility).

B. Breast-fed infants have 50 times the daily recommended intake of dioxins and PCBs per kilogram of body weight than adults. Children whose mothers had higher amounts of chemicals in their bodies have lower neurological, hormonal and immunological performance than children whose mothers were at the low end of chemical exposure.

C. In a study looking at prostate cancer mortality among pesticide applicators, it was concluded that there were twice as many deaths as would normally be expected for this population of individuals (standardized by race and age). There were 1,874 deaths to 33,658 licensed pesticide applicators during 1975-1993.

D. Between 1982 and 1996 there has been a substantial increase in the number (1.6 million) and percent (21.4%) of women with impaired fertility. The greatest increase was seen in women under 25 (41.9%).

E. Researchers examined 34 Chinese men working in a pesticide factory and 44 in a textile factory. They found that the workers with high exposure to organophosphate pesticides showed significant reproductive abnormalities, including decreased testosterone levels.
3. **Observation:**

A. While examining 20 years of scientific literature on wildlife populations near the Great Lakes, a researcher noticed many physical abnormalities, higher death rates in the young, and decreasing population in many kinds of animals, especially top-of-the-food-chain predators. Subsequent tests of tissue samples indicated high levels of synthetic chemicals.

B. It was observed that male fish in certain urbanized freshwater streams in England in the 1980's had gross abnormalities of the gonads and disruption of normal male spawning behavior. These male fish were highly feminized (both male and female reproductive structures). Laboratory tests revealed high levels of the egg yolk protein vitellogenin in their blood. Vitellogenin, normally absent in males, is now viewed as a specific biomarker for the exposure of male fish to feminizing environmental chemicals.

4. **Laboratory Experiments:**

The measurement of vitellogenin levels in male fish exposed to synthetic chemicals has now been established as a useful and informative screening for the potential environmental impact of new drugs, pesticides, plasticizers, and other industrial substances.

A. Pyrethroid insecticides, commonly used for indoor pest control were applied to a human breast cell line (cultures of cells from human breast tissue). The treated cells proliferated (divided) at a very rapid rate in a dose-response fashion. These studies indicate that pyrethroids should be considered to be hormone disruptors.

B. Red-eared slider turtles have temperature-dependent sex determination. Eggs incubated at 28.6 C typically result in male hatchlings. Three-hundred freshly laid eggs were kept at 28.6 degrees C in the laboratory, and a range of doses of 17B-estradiol (form of estrogen) was applied to the shells. The eggs receiving the lowest dose (400pg/egg) had a rate of 14.4% sex reversal.

C. Captive female mink were fed a mixture of 16 aryl methly sulfones for one year, then were mated after nine months. The exposed group had
more kits per litter and the kits had a lower mean birthweight and lower survival at two weeks (47% exposed compared to 73% controls). Both mothers and 5-week old kits accumulated the aryl methyl sulfones in the muscle and liver.

D. It was observed that a large number of frogs living in a Minnesota pond had widespread deformities. The pond water was collected, and brought into a lab. Very young frog embryos were exposed for 96 hours to the pond water, and they developed deformities. The pond water was filtered through activated carbon and the health problems were eliminated.

REFERENCES:


Environmental Microarray
And Biomarkers

In recent years, some scientists have proposed that chemicals might inadvertently be disrupting the endocrine system of humans and wildlife. There is mounting concern in the scientific, environmental, private and governmental sectors concerning a wide range of substances, known as endocrine disruptors, that have the potential to interfere with the normal functioning of a living organism’s hormone system. Endocrine disruption has the potential to compromise proper development in organisms, leading to reproductive, behavioral, immune system and neurological problems, as well as the development of cancer.

A variety of chemicals have been found to disrupt the endocrine systems of animals in laboratory studies, and compelling evidence shows that endocrine systems of certain fish and wildlife have been effected by chemical contaminants, resulting in developmental and reproductive problems. However, the relationship of human diseases of the endocrine system and exposure to environmental contaminants is poorly understood and scientifically controversial. Effects often do not show up until later in life.

Scientists have identified dozens of human made chemicals that can disrupt an animal’s endocrine system -- a group of organs that release chemicals into the body that are important for a wide range of activities -- including growing, managing stress and reproducing. One of the chemicals released by the endocrine system is estrogen. Estrogen helps females develop into adults with the ability to reproduce -- it helps prepare the body for pregnancy -- and is active during pregnancy.

Some human made chemicals can act like estrogen in an animal's body. These so called "estrogen mimics" are being increasingly detected in agricultural runoff, effluent from water treatment plants often from drugs flushed down the toilet in homes and pollution from manufacturers including plastics factories and paper pulp mills. These chemicals are also turning up in aquatic animals and birds that live in or near streams, rivers and the ocean. They are known -- in certain concentrations -- to disrupt the ability of alligators, frogs, birds and fish to mature and reproduce.
There are various theories about how estrogen mimics disrupt an animal's endocrine system. In one theory, the chemicals bind to a site on cells within the animal that normally only stick to estrogen. Somehow, filling up these sites with chemicals that weren't produced by the body can lead to males producing eggs instead of or in addition to sperm. It can cause an individual to act more like the opposite gender.

A biomarker is a biological response to an environmental chemical, which gives a measure of exposure and sometimes also a toxic effect. The biological responses may be at the molecular, cellular or whole organism level.

There is increasing evidence that many xenobiotic chemicals (called as endocrine disruptor chemicals, EDCs) through interfering with the endocrine system, have the capability to induce developmental and reproductive abnormalities in humans and animals. The yolk protein precursor vitellogenin (Vtg) has proved to be a simple and sensitive biomarker for assessing exposure of fish to EDCs, especially the estrogenic compounds.

Vitellogenin (Vg) is an egg yolk precursor protein expressed only in female fish and is normally dormant in male fish. However, when male fish are exposed to estrogenic EDCs the Vg gene is expressed in a dose dependent manner. Hence, Vg gene expression in male fish has been used as a molecular marker of exposure to estrogenic EDCs.

Other biomarkers of endocrine disruption that can be found in the blood serum of male fish including two forms of vitellogenin (vitellogenin-1 & vitellogenin-2) and two forms of another egg protein, choriogenin (choriogenin-L & choriogenin-H).

These biomarkers can be identified by analyzing the messenger RNA (mRNA) in the cells of the test organism. This is done by creating cloned DNA (cDNA) from the mRNA and using a microarray to identify the cDNA.

In this experiment you will analyze 8 fish (identified as males) that were captured from 8 environmentally sensitive locations: 1) downstream from a paper mill, 2) downstream from a sewage plant, 3) downstream from a nuclear reactor, 4) downstream from an oil refinery, 5) downstream from a cattle farm, 6) downstream from landfill, 7) downstream from agricultural runoff and 8) downstream from a mining operation.