STAR REPORT

U.S. EPA Office of Research and Development's Science To Achieve Results (STAR) Research in Progress

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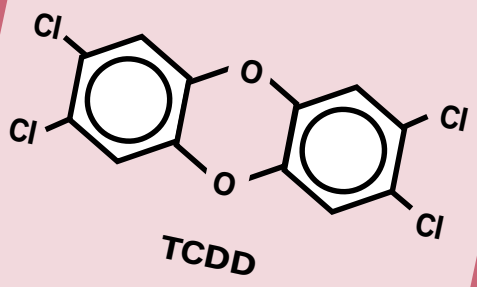
THE ENDOCRINE DISRUPTOR PROBLEM

In recent years there have been reports of wildlife with reproductive disorders, deformities and other developmental disorders caused by environmental chemicals that affected the animals' endocrine systems. The endocrine system is a complex of organs, tissues and hormones in humans and other living things. The system is responsible for maintaining normal reproduction, development and other aspects of "homeostasis" (maintaining equilibrium of the body's chemistry and other condition factors). Substances that interfere with these processes are called "endocrine disruptors".

Endocrine disruption problems have been identified primarily in wildlife and laboratory animals exposed to relatively high concentrations of some chemicals, most of which are man-made environmental contaminants, but some of which occur naturally. These include organochlorine pesticides and breakdown products, industrial chemicals such as polychlorinated biphenyls (PCBs) and organometals, waste chemicals such as dioxins, a few plastics-related chemicals such as bisphenol, nonaphenols and phthalates, synthetic hormones and natural plant-derived hormones. Whether effects are occuring in humans, or whether wildlife frequently incur harm from environmental concentrations of these substances is not yet known, although conditions in some locations indicate cause for concern. While some studies find declines in the quantity and quality of sperm production in humans over the last four decades, other studies show no decrease. Reported increased incidences of human cancers in organs of the reproductive and hormonal systems (breast, testes, prostate) have led to speculation that this could be related to environmental endocrine disruption. And correlational evidence from many areas indicates that some populations of birds, fish, reptiles and mammals have been harmed by environmental contaminants affecting their endocrine systems.



TCDD

Some endocrine disruptor research focuses on the ways these chemicals interfere

Because the endocrine system plays a critical role in growth, development and reproduction, even small disturbances in endocrine function can have profound and lasting effects.

Many of the chemicals associated with endocrine disruption, such as the pesticide DDT and the industrial compounds PCBs, have been banned for U.S. manufacture and new use for a number of years. However, they remain in the U.S. environment because of residual contamination, or possibly because of illegal use or disposal, or air, water or human transport across international boundaries. Another important question is the extent to which chemicals currently in production and use may be acting as endocrine disruptors of wildlife or humans in the United States.

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with reproduction, including the production of normal germ cells (eggs and sperm). In addition, it is known that impacts of endocrine disturbances in humans or animals can be particularly damaging if exposure occurs during the highly sensitive prenatal and early postnatal periods. Small changes in endocrine status during gestation can have delayed consequences that are evident much later in adult life or in a subsequent generation. Consequently, other studies are targeted towards understanding effects of exposures during pregnancy.

THE NATIONAL ENDOCRINE DISRUPTORS RESEARCH INITIATIVE

Because of the potential for serious consequences to humans or wildlife, the United States has joined with other nations to conduct laboratory and field research of many types to investigate whether human health is being threatened by endocrine disruptors in the environment, and whether environmental harm to wildlife is more widespread than had previously been understood. A coordinated research effort is being led in the U.S. by the National Science and Technology Council (NSTC) Committee on Environment and Natural Resources. The NSTC is a cabinet-level council chaired by the President that serves as the principal means for coordinating science and technology issues across the Federal government.

This report summarizes academic research into endocrine disruptors supported by the U.S. Environmental Protection Agency (EPA). This work is supported through EPA's principal external scientific research program, the Science to Achieve Results (STAR) program. Other federal agencies also support academic research on endocrine disruptors. In addition, EPA,

General Information: The Environmental Protection Agency's STAR Research Program

Grants described in this report are part of EPA's Science to Achieve Results (STAR) program, a major research initiative designed to improve the quality of scientific information available to support environmental decision making. The STAR program is managed by EPA's National Center for Environmental Research and Quality Assurance in the Office of Research and Development (ORD). The program funds approximately 200 new grants every year, with the typical grant lasting three years. Funding levels vary from $50,000 to over $500,000 per year, with FY 1997 funding level at about $80 million for grants to individual principal investigators or groups of investigators. Additional STAR funds are provided for a number of Research Centers specializing in scientific areas of particular concern to EPA, and for a fellowship program supporting graduate students conducting environmental research.

the National Institute of Environmental Health Sciences (NIEHS), the Department of the Interior and other agencies are conducting research addressing the national agenda in their own laboratories. Information on the overall body of research coordinated through the NSTC Endocrine Disruptors Research Intiative (EDRI) can be found at EDRI and EPA sites on the World Wide Web, or by mail, at websites and addresses listed at the end of this report.

Research Areas Particularly Supported by EPA

EPA supports endocrine disruptor research primarily in the following areas:

- ◊ Methods to monitor and characterize exposure of humans or wildlife to endocrine disruptors;
- ◊ Models to estimate exposure to endocrine disruptors from different sources and multiple pathways;
- ◊ Development of biomarkers of endocrine disruptor exposure and effects;
- ◊ Development and validation of test systems to screen for chemicals with specific mechanisms of action that affect different endocrine pathways; particularly methods that are applicable to many types of living organisms;
- ◊ Development of toxicological models describing how particular chemicals act to interfere with endocrine systems, based on species-specific characteristics, with particular emphasis on models that can extrapolate effects from one animal species to another, or to humans; and
- ◊ Methods and models that relate effects at subcellular levels to effects in individual humans or animals, or in human or animal populations.

ENDOCRINE DISRUPTOR RESEARCH SUPPORTED BY EPA'S "STAR" PROGRAM

Research Concerning Impacts on Humans

One of the few well-documented cases of widespread human exposure to chemicals that may mimic the female hormone estrogen, and thus may cause endocrine disruption, was a 1973 incident in Michigan, in which cattle feed was contaminated by polybrominated biphenyls (PBBs). After it was determined that many people had been exposed to meat and dairy products from contaminated cattle, the Michigan Department of Community Health began to gather public health data on the exposed human population. This included data on people's blood levels of PBBs three years after the exposure. PBBs are of concern because chemically similar compounds, such as PCBs, are known to impair female reproductive development. A grant from the EPA STAR program has been awarded to **Emory University** to assess whether there have been any health effects in Michigan in exposed women or their daughters. Data reviewed will include reproductive defects or diseases, thyroid dysfunctions, infertility or other endocrine-related effects. The findings concerning any effects in humans, together with data on known exposure to a contaminant, will be invaluable in assessing potential health risks from exposures in other circumstances.

In an important federal/academic research partnership, EPA is providing additional support to investigators at the **University of Missouri** who participate in the Food and Drug Administration's program to develop an Estrogen Knowledge Base (EKB). The EKB program incorporates a wealth of data on observed health impacts in humans and animals with information on theoretical impacts. Theoretical information is developed through a statistical modeling

technique called the "Quantitative Structure Activity Relationships" approach. The new EPA grant will allow investigators to expand the database to better establish quantitative relationships among effects in various animals and in humans. This will expand the number of chemicals for which we can obtain sound estimates of the likelihood of adverse health effects. This will help EPA and the Food and Drug Administration more effectively target their own screening and testing programs, and those they require chemical manufacturers to conduct.

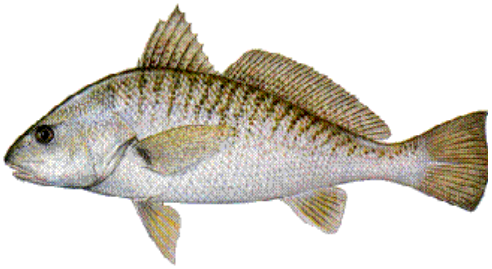
Research into Effects on Wildlife

The alligators of Florida's Lake Apopka have serious reproductive failures that have been attributed to endocrine disruptors. Many of the alligators are sterile, and abnormalities such as underedeveloped penises, abnormal testes and abnormally high levels of estrogen are common. Known endocrine disruptors found in the lake at levels of concern include the banned pesticides dieldrin, toxaphene and DDT, and DDT's breakdown product DDE. The **University of Florida** has received a STAR grant to study the condition of Lake Apopka alligators and their eggs. Researchers will investigate mechanisms by which the chemicals may take effect, including direct disruption of sex hormones, developmental effects before and after hatching, and the additional possibility that harm to immune systems increases susceptibility to disease. In a related study, **Texas Tech University** will compare Lake Apopka data to the conditions of crocodiles in lagoons in Belize, Central America, some of which are contaminated with DDE. A full analysis will be done of chemicals present in each lagoon, and of whether endocrine disruption is occuring in the crocodiles. This study is important in supporting risk assessment for the Belize ecosystem, as well as helping to establish the relative severity of Lake Apopka impacts.

Florida's **Mote Marine Laboratory** is leading a team of investigators from Florida and Oregon in a study of the causes of infertility in Bonnethead sharks in the Tampa Bay area. Female sharks are frequently retaining unfertilized rather than fertilized eggs following mating, either due to abnormal sperm production in males, or damage to sperm stored in females. There may be other impairments as well. Hypotheses include the possibility that organochlorine pesticides are the cause. A full range of mechanisms will be assessed by evaluating tissues, cells, immune systems and hormone levels.

A particularly severe case of ecosystem contamination was the manufacturer's release

of DDT in the 1960s and '70s to the Southern California sewer system, which discharges to the sea. The near extinction of brown pelicans in the area was widely reported, and fish and other animals also experienced reproductive disorders. Since the release ended and DDT was banned, the pelican population has



recovered, but contamination remains in marine sediments and fish. The **University of Texas** is conducting a laboratory study to try to elucidate possible endocrine disruption mechanisms in fish exposed to endocrine disruptors that mimic combinations of DDTs and DDEs found in California fish tissues.The study will determine mechanisms of impairment to gonads, other organs and embryos. Fish used will be a well-established laboratory test species, a croaker, not native to California. However, investigators feel any findings will be sufficiently generalizable that they would be relevant to risk assessments for the California fishes, as well as to species in other locations.

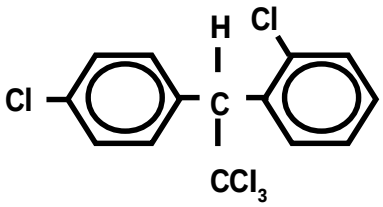
Many birds, including all songbirds, are "altricial" species whose chicks are incompletely developed at hatching, requiring weeks for full development of brains, reproductive systems and other organs. We know that endocrine disruptors such as DDT and DDE cause reproductive damage in some species such as bald eagles, osprey and pelicans. These chemicals are "xenoestrogens", estrogen-like chemicals found in the environment. They are also anti androgenic, interfering with hormones involved in male development and reproduction. The **University of California at Davis** has received a STAR grant to study effects of xenoestrogen exposure on hormonal processes and development of altricial chicks.

Research Using Laboratory Animals as Models of Potential Risk to Humans or Wildlife

The **University of Missouri** is studying effects of the pesticide methoxychlor, an environmental estrogen, on mice whose characteristics make them a good model to assess how endocrine disruptors act in other animals and humans. Such mechanis-

tic animal studies can help in developing treatments or interventions to protect human health. The university received a grant from NIEHS for research with a mouse strain lacking a functional estrogen receptor gene. Comparing effects of exposure in these and normal mice will reveal whether certain non-estrogen receptor proteins are involved in responses to methoxychlor. The University has also received an EPA STAR grant to clone the gene believed to serve as the methoxychlor receptor. This cloned gene is needed to fully investigate whether the methoxychlor receptor mechanism operates in the way hypothesized by these investigators.

ronment. The dioxin TCDD, a potent endocrine disruptor, and other dioxins and dibenzofurans, are waste products of a number of industrial processes, including chlorine-using processes and waste combustion. The **University of Kansas** is studying ovarian disorders and other effects caused by exposing rats to TCDD, other dioxins, furans and PCBs. Preliminary data indicate that direct ovarian damage, rather than indirect effects from influences on organs such as the pituitary, is one reason endocrine disruptors impair female reproduction. In a study of another aspect of female reproduction, the enzymes responsible for



o,p - DDT

gestation are longer than in rodents, and thus more like humans, **Colorado State University** is investigating effects of DDT and the pesticide vinclozolin in male rabbits. Sub-cellular changes and abnormalities in levels of pituitary and sex hormones will be among the mechanisms investigated. Another

It is known that impacts of endocrine disturbances in humans or animals can be particularly damaging if exposure occurs during the highly sensitive prenatal

A STAR grant to study PCB effects in rats has been awarded to **Mississippi State University.** Questions to be asked include: 1) whether organ dysfunctions are caused by mechanisms involving reduced blood or liver levels of sex steroids; and 2) whether such effects occur at exposures that might occur in the environment. The dioxin TCDD, a potent endocrine disruptor, and other dioxins and dibenzofurans, are waste products of a number of industrial processes, including chlorine-using processes and waste combustion. The **University of Kansas** is studying ovarian disorders and other effects caused by exposing rats to TCDD, other dioxins, furans and PCBs. Preliminary data indicate that direct ovarian damage, rather than indirect effects from influences on organs such as the pituitary, is one reason endocrine disruptors impair female reproduction. In a study of another aspect of female reproduction, the enzymes responsible for maintaining healthy endometrial condition, **Vanderbilt University** is using cell cultures, corroborated by mouse experiments, to evaluate the hypothesis that TCDD may interfere with normal regulation of these hormones.

To study impacts in a mammal whose life span and study of male reproductive system impacts uses dogfish sharks. This species is of ecological importance in its own right, and is a model of possible effects in other animals or humans. Investigators at **Boston University** will expose male sharks to a number of xenoestrogens. Objectives are to understand

genetic or cellular mechanisms by which the chemicals act; to seek molecular markers that indicate that an animal has been exposed; and to document health impacts such as sterility or testicular cancer. **Duke University** is using zebrafish embryos for a related study. Advanced gene scanning techniques will characterize the structure and location of estrogen receptor RNA, to increase understanding of how genes act in response to environmental estrogens.

The **University of California at Davis** is assessing effects of two chemicals, one a xenoestrogen, and one an anti-androgen, on males and females of a fish called *medaka.* Potential impacts include gonad and liver damage, disturbed mating behavior, abnormal sperm or eggs, and low survival of the young.

One animal study uses a bird as the model species. The **University of Maryland** will work with Japanese quail to assess when in their life cycle susceptibility to endocrine disruption is the greatest. Impacts on gonads and other glands, including the hypothalamus and pituitary, will be assessed. This species is used because it is a good laboratory subject with an exceptionally well documented reproductive cycle and physiology. Results will be relevant to a range of bird species.

Some endocrine disruptors are natural substances. **Duke University** has received a grant for work using laboratory rats to assess whether some phytoestrogens, compounds that occur naturally in plants, could potentially have endocrine disrupting effects at concentrations that could occur in human diets. This study will look for any impacts from soy phytoestrogens on the rats' brain or reproductive system development, mating or related behaviors such as adolescent behavior patterns.

Developing Screening Procedures for Endocrine Disruptors

In an effort to develop a practical screening procedure for endocrine disrupting effects in aquatic vertebrates, the **University of Alabama** is leading a project to refine a mosquitofish test system. A range of chemicals will be tested and readily observed effects on developmental abnormalities and blood chemistry will be defined as screening procedures. **North Carolina State University** is working under a STAR grant to develop routine testing procedures for potential endocrine disruptors, using water fleas as a model for other aquatic invertebrates.

Find Out More About the STAR Research Program

For further information about the National Endocrine Disruptors Research Initiative is available at the following Internet websites:

The Endocrine Disruptor Research Initiative website of the NSTC Committee on the Environment and Natural Resources: **www.epa.gov/endocrine/edrifact.html**

EPA's Endocrine Disruptor Screening and Testing Advisory Committee website: **www.epa.gov/opptintr/opptendo/index.htm**

General information on EPA's STAR research program is available from the following sources:

ORD's National Center for Environmental Research and Quality Assurance (NCERQA): Internet website: **http://www.epa.gov/ncerqa**

Mailing Address:

Office of Research and Development
National Center for Environmental Research and Quality Assurance
Office of the Director (8701 R)
401 M Street, SW
Washington, DC 20460

STAR Research Projects Described in this Report

1996 STAR Awards

Duke Medical Center, Examination of the Estrogen Response Pathways in a New Vertebrate Model

U. California-Davis, Critical Stages in Avian Development: Estrogenic Hazards to Altricial and Precocial Birds

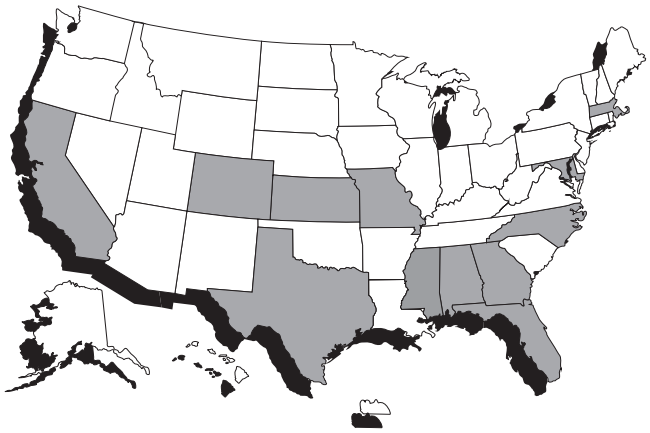
Mississippi State University, Biochemical and Reproductive Effects of Gestational/Lactational Exposure to PCB's with Respect to Endogenous Sex Steroids and the Proestrogen, Methoxychlor

U. California-Davis, An *in Vivo* Model for Detection of Reproductive Effects of Endocrine Disruptors

Duke University, Developmental Effects of Dietary Soy Phytoestrogens

Emory University, The Michigan PBB Cohort 20 Years Later: Endocrine Disruption?

Boston University, Xenoestrogen Effects During Premeiotic Stages of Spermatogenesis: Development of an *in Vitro* Test System and Molecular Markers of Action



U. Missouri-Columbia, Methoxychlor and Environmental Estrogen Receptors in ER-Minus Mice

1997 STAR Awards

Colorado State University, Anti-androgenic Pesticides: Impact on Male Reproduction

Duke University, Developmental Effects of Dietary Soy Phytoestrogens

Mote Marine Laboratory, The Mechanisms and Effects of Endocrine Disruption on Infertility in the Bonnethead Shark on Florida's Gulf Coast

North Carolina State University, Metabolic Androgenization of Invertebrates by Endocrine-Disrupting Chemicals

Texas Tech University, Exposure and Response of Morelet's Crocodile (Crocodylus moreletii) Populations to Endocrine Disrupting Compounds in Belize, Central America

U. Alabama - Birmingham, A Short-Term *in Vivo* Screening System for Endocrine Disruptors Utilizing Mosquito-Fishes (Gambusia affinis or G. holbrooki)

U. California - Davis, Environmental Endocrine Disruption in Avian Wildlife

University of Florida, Endocrine Disruptors and Host Resistance in Lake Apopka Alligators

U. Kansas Medical Center, Endocrine Disruptors: Effects on the Thyroid

U. Kansas Medical Center, Models Assessing Direct Effects of Dioxins and Related Compounds on the Ovary

U.Maryland - College Park, Critical Stages in Sublethal Exposure to EDCs in a Quail Model System

U. Missouri - St. Louis , Computational Tools for the Prediction and Classification of Estrogenic Compounds

U. Texas - Austin, Reproductive and Endocrine Effects of o,p'-DDT, an Environmental Estrogen, and p,p'-DDE, an Antiandrogen, in Male and Female Atlantic Croaker during Critical Periods of their Reproductive Life History Cycles

Vanderbilt University, Dioxin and Steroid Regulation in an Endometriosis Model

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