Environmental Protection Agency
Endocrine Disruptor Screening Program

Report to Congress

August 2000

EDSP Report to Congress

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Executive Summary

In 1996, through enactment of the Food Quality Protection Act which amended the Federal Food, Drug and Cosmetic Act, Congress directed the Environmental Protection Agency to develop a screening program to determine whether certain substances may have hormonal effects in humans. There is concern that certain pesticide chemicals and other substances may modify the normal functioning of human and wildlife endocrine, or hormone, systems and cause developmental, behavioral, and reproductive problems.

EPA chartered a scientific advisory committee under the Federal Advisory Committee Act to provide advice and recommendations on a strategy for determining whether substances may have an effect similar to the effects produced by naturally occurring hormones. The advisory committee, known as the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), recommended that EPA address effects on both humans and wildlife, examine effects on biological processes involving the estrogen, androgen, and thyroid hormones, and include pesticide chemicals, commercial chemicals, and environmental contaminants within the scope of the program. Following the recommendations of the EDSTAC, EPA announced the establishment of the Endocrine Disruptor Screening Program (EDSP) in August 1998.

EPA will use a multi-step, or "tiered," approach for determining whether a chemical substance may have an effect similar to that produced by naturally occurring hormones. The tiered approach will consist of sorting chemicals based on existing, scientifically relevant information, and prioritizing chemicals to determine which substances should be evaluated before others. Following these steps, Tier 1 screening will identify substances which have the potential to interact with the endocrine system, and Tier 2 testing will confirm that potential and characterize the effects. In establishing priorities, EPA will use appropriate available exposure information.

Implementation of the EDSP is currently proceeding on two fronts. First, EPA is establishing a method for setting priorities for screening. For commercial chemicals and environmental contaminants other than pesticides, this method will include use of a database and software which EPA is developing. Pesticide active ingredients will be prioritized separately from other chemicals because there are generally more data available on the health and environmental effects of these substances.

As part of the screening process, EPA has completed a feasibility study on the high throughput pre-screening (HTPS) process to evaluate this method's ability to indicate whether a chemical has the potential to interact with the endocrine system. The feasibility study showed that the HTPS process required further development before routine use. EPA is consequently examining other methods to serve this screening function, including a Quantitative Structure-Activity Relationship (QSAR) computer simulation model to screen chemicals for their ability to interact with the endocrine system based on the molecular structure of the substance.

Second, EPA is ensuring that the Tier 1 and Tier 2 assays are scientifically validated. Validation consists of developing protocols to conduct specific assays, evaluating their effectiveness, and ensuring that the assay can be performed reliably and consistently in different laboratories. At the present time, there are no adequately validated assays for determining if a

substance may have an effect in humans that is similar to an effect produced by a naturally occurring hormone. All of the EPA validation work is being conducted in close liaison with the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) established by the National Toxicology Program, under the auspices of the National Institute of Environmental Health Sciences (NIEHS), and is following the ICCVAM principles.

Following validation of the assays, EPA plans to publish final test guidelines and a federal register notice setting forth the final policy and procedures for implementation of the EDSP. EPA will publish a proposed list of chemicals for Phase I screening approximately one year in advance of the date screening would be required to begin. EPA anticipates requiring screening of pesticide active ingredients and other pesticide formulation ingredients with high production volume beginning in 2003.

In order to fully evaluate the potential risk to humans and wildlife of exposure to endocrine disruptors, EPA intends to collect data beyond those developed through its screening program. Specific research gaps have been identified by EPA, the National Academy of Sciences, and the Committee on Environment and Natural Resources (CENR) of the National Science and Technology Council. Much of the recommended research is underway or is planned by federal agencies as part of the Endocrine Disruptor Research Initiative developed under the auspices of the CENR. EPA is committed to minimizing the number of animals that will be used in implementing the EDSP, and to incorporating alternative test methods whenever and wherever possible.

EPA recommends continued Congressional support for implementation of the EDSP and of endocrine disruptor research.

Introduction

When Congress amended the Federal Food Drug and Cosmetics Act (FFDCA) in the Food Quality Protection Act (FQPA) of 1996, it directed the U.S. Environmental Protection Agency (EPA) to develop a screening program to determine whether certain substances may have hormonal effects in humans.[1] The statute covers effects similar to those produced by a naturally occurring estrogen, or such other endocrine effects as the EPA Administrator may designate. Congress directed EPA to develop this program using appropriate validated test systems and other scientifically relevant information not later than two years after August 3, 1996, in consultation with the Secretary of the Department of Health and Human Services.

[1]In 1996, Congress also amended the Safe Drinking Water Act and gave EPA authority to provide for the testing, under the FFDCA Screening Program, "of any other substance that may be found in sources of drinking water if the Administrator determines that a substantial population may be exposed to such substance." (42 U.S.C. § 300j-17.)

EPA established a multi-sector scientific advisory committee, under the Federal Advisory Committee Act (FACA), to obtain the best advice available on how to design the program. After receiving the committee's recommendations in August 1998, EPA developed the Endocrine Disruptor Screening Program (EDSP) by the congressional deadline, setting forth the basic components of the program in an August 1998, Federal Register notice (63 Fed. Reg. 42852). EPA published another Federal Register notice in December 1998, that provided additional details and an opportunity for public comment on its EDSP (63 Fed. Reg. 71542).

After obtaining public comment and review of the program by a joint subcommittee of FIFRA's Scientific Advisory Panel and the EPA's Science Advisory Board, EPA began implementing the program by August 3, 1999, according to the schedule established in the statute. EPA's implementation of the Endocrine Disruptor Screening Program currently is proceeding on two fronts: 1) the Agency is finalizing the tools and processes that will be used to establish priorities for screening in the Program; and 2) EPA is ensuring that the scientific tests, which are part of the Program, are validated as required by statute.

In this report, EPA responds to Congress's specific requests for information on:

- **the findings of the Administrator resulting from the screening program;**
- **recommendations for further testing needed to evaluate the impact on human health of the substances tested under the screening program; and**
- **recommendations for any further actions that the Administrator has determined are appropriate based on the findings.**

EPA first provides an overview of the endocrine disruptor issues and describes its Endocrine Disruptor Screening Program. We then describe progress in implementing the program. Finally, we present EPA's ongoing research relating to endocrine disruptors and the measures the Agency is taking to address animal welfare concerns under the EDSP.

Overview of the Endocrine Disruptor Issue

There is concern that certain pesticide chemicals and other chemical substances, as well as certain naturally-occurring substances such as phytoestrogens[2] in foods, may modify the normal functioning of human and wildlife endocrine, or hormone, systems. Endocrine disruptors (also referred to as hormonally active agents) may cause a variety of problems with, for example, development, behavior, and reproduction. They have the potential to impact both human and wildlife populations (US EPA, 1997; NAS, 1999).

[2]The EDSTAC recommended that screening and, if necessary, testing be considered for phytoestrogens and mycotoxins in order to assess the potential additive, antagonist, and synergistic effects of these substances with other hormonally active chemical substances . (EDSTAC, 1998).

Although many pesticides, and some industrial chemicals, may have already undergone extensive toxicological testing, conventional toxicity tests may be inadequate to determine whether these substances interact with specific components of the endocrine system and whether additional testing is needed for the EPA to assess and characterize more fully their impact on both human and ecological health. Scientific knowledge related to endocrine disruptors is still evolving; however, there is widespread scientific agreement that a screening and testing program would be useful in elucidating the scope of the problem. (EDSTAC, 1998; EPA, 1999; NAS, 1999).

An endocrine system is found in nearly all animals, including mammals, non-mammalian vertebrates (e.g., fish, amphibians, reptiles, and birds), and invertebrates (e.g., 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.

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, such as birth control pills, are used to cause some of these effects intentionally.

A variety of effects on humans and wildlife have been attributed to endocrine disruptors (US EPA, 1997; NAS, 1999). Although there is controversy on the subject, EPA (US EPA, 1997) and the National Academy of Sciences (NAS, 1999) published recent reports based on reviews of the scientific literature on studies of declining human sperm counts over the last fifty years. Wildlife have been reported with malformed genitalia, aberrant mating behavior, sterility, and other physical and behavioral anomalies (US EPA, 1997; NAS 1999). A difficulty in attributing specific health effects to specific chemicals is that we do not currently know which chemicals may interfere with endocrine system function, the extent to which problems exist, or how widespread they may be in the environment. Nonetheless, in view of existing data, endocrine disruptors warrant further study (US EPA, 1997; NAS, 1999). The agency has, therefore, initiated a two-phased implementation strategy for its Endocrine Disruptor Screening Program: Standardization and validation of screens and tests in accordance with statutory mandates of the FFDCA; and a research program directed toward reducing uncertainty in this complex and scientifically controversial area.

Overview of EPA's Endocrine Disruptor Screening Program

The Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC)

Recognizing the expertise available outside the Agency on endocrine disruptor issues, as well as the evolving nature of the science surrounding endocrine disruption, EPA chartered an

advisory committee under the Federal Advisory Committee Act to advise the Agency on developing a program to comply with the FFDCA section 408(p) requirements.

The Advisory Committee, known as the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), was comprised of members representing the commercial chemical and pesticide industries, federal and state agencies, worker protection and labor organizations, environmental and public health groups, and research scientists. EPA charged the EDSTAC with providing advice and recommendations to the Agency regarding a strategy for testing chemical substances to determine whether they may have an effect in humans similar to an effect produced by naturally occurring hormones. Specifically, EPA requested the committee to:

- Develop a flexible process to select and prioritize chemicals and pesticides for screening, recognizing the need to obtain and utilize appropriate exposure information in setting priorities;

- Develop a process for identifying new and existing screening tests and mechanisms for their validation;

- Agree on a set of available, validated screening tests for early application; and

- Develop a process and criteria for deciding when additional tests, beyond screening tests, are needed and how any of these additional tests will be validated.

In response to this charge, the EDSTAC reached consensus on a set of recommendations for the Agency. These recommendations are contained in the EDSTAC Final Report, available on the EPA web site at *www.epa.gov/scipoly/oscpendo/history/*. Based on the body of available scientific information available at that time, the EDSTAC recommended that EPA's Endocrine Disruptor Screening Program (EDSP) go beyond the FFDCA statutory minimum to address effects on both humans and wildlife; examine effects to hormone-related processes for the estrogen, androgen and thyroid hormones; and include chemical substances and representative mixtures that are not pesticides. The screening and testing assays would only be conducted in laboratories around the country.

Considering the EDSTAC's diverse membership, EPA found its consensus recommendations compelling. More importantly, EPA found the advice contained in the EDSTAC Final Report scientifically rigorous. As such, EPA relied heavily on EDSTAC's advice and recommendations in developing the EDSP.

Endocrine Disruptor Screening Program Proposed Statement of Policy

EPA first set forth the basic components of the EDSP in the August 11, 1998, Federal Register (63 Fed. Reg. 42852), and offered additional detail in the December 28, 1998 Federal Register (63 Fed. Reg. 71542). After careful consideration of the EDSTAC's recommendations, the EPA proposed that the EDSP's scope include:

- ***Effects on Humans and Wildlife***

Adverse effects on wildlife and fish can serve as an early warning of potential health risks for humans. There is strong evidence for endocrine disruption observed in natural wildlife and fish populations. Moreover, wildlife and fish are inherently valuable components of ecosystems, and they act as sentinels for the relative health of the environment that they share with humans. (63 Fed. Reg. 71545.)

- ***Effects on estrogen, androgen, and thyroid (EAT) hormone-related processes***

These three hormone systems are presently among the most studied of the approximately 50 known vertebrate hormones. *In vitro* (test tube) and *in vivo* (whole animal) test systems to examine estrogen, androgen, and thyroid (EAT) hormone related effects exist, and are currently the most amenable for regulatory testing. Further, including EAT effects will cover aspects of reproduction, development, and growth. EPA recognizes that there is a great deal of ongoing research related to other hormones and test systems. As more scientific information becomes available, EPA will consider expanding the scope of the EDSP to other hormones. For now, however, the EAT effects and test systems represent a scientifically reasonable focus for the Agency's EDSP. (63 Fed. Reg. 71545.)

- ***Evaluation of chemical substances***

The chemicals to be prioritized for endocrine disruptor screening and testing include pesticide chemicals, commercial chemicals, and environmental contaminants. Commercial chemicals and environmental contaminants are being included because chemicals like PCB's and other non-pesticidal chemicals have been implicated as endocrine disruptors. EDSTAC believed that substances such as drugs, cosmetics, and nutritional supplements are known to contain naturally occurring estrogens and should be screened for their endocrine disruption potential. Although EPA will work with the US FDA and other federal partners, EPA has no regulatory purview over drugs, cosmetics, and nutritional supplements unless they occur as environmental contaminants.

EPA will use a multi-step, or "tiered," approach for determining whether a substance may have an effect in humans, fish, and wildlife that is similar to an effect produced by naturally occurring hormones. The core elements of the tiered approach consist of sorting, priority setting, Tier 1 screening, and Tier 2 testing (Figure 1).

Sorting: Chemicals will undergo sorting into four categories based on existing, scientifically relevant information:

- Category 1 will consist of chemicals with sufficient, scientifically relevant information to determine that they are not likely to interact with the estrogen, androgen or thyroid systems. Currently, EPA believes that it is appropriate to assign certain polymers and certain exempted chemicals, into this category;
- Category 2 will consist of chemicals for which there is insufficient information to determine whether or not they are likely to interact with the estrogen, androgen or thyroid systems and which, therefore, need Tier 1 screening data to enable EPA to make this determination;
- Category 3 will consist of chemicals with sufficient screening data to indicate endocrine activity but with inadequate data to characterize actual effects and which, therefore, will be queued for Tier 2 testing;
- Category 4 will consist of chemicals for which there are already sufficient data for EPA to perform a hazard assessment.

Priority Setting: After sorting, the vast majority of chemicals will fall into Category 2 (chemicals with insufficient information to determine whether or not they interact with the endocrine system). There are very few data on a large number of chemicals and most of the data are disparate. Because there are existing data on the pesticide active ingredients, the Agency will prioritize pesticides in a process separate from pesticide formulation ingredients and other commodity chemicals. For the latter two groups of chemicals, EPA has established a "compartment-based" priority setting approach based on the three main categories for organizing information and criteria related to priority setting: Exposure; Effects; and Statutory Criteria. The exposure category includes exposure through water (including surface water, groundwater, and drinking water). The priority setting processes are discussed in more detail in the section of the Report entitled "Implementation Progress" (see page 8, below).

Tier 1 Screening: The purpose of Tier 1 screening is to identify substances that have the potential to interact with the endocrine system. To ensure that Tier 1 screening is effective, the screening battery includes known endocrine disruptor mechanisms for the estrogen, androgen and thyroid hormone systems.

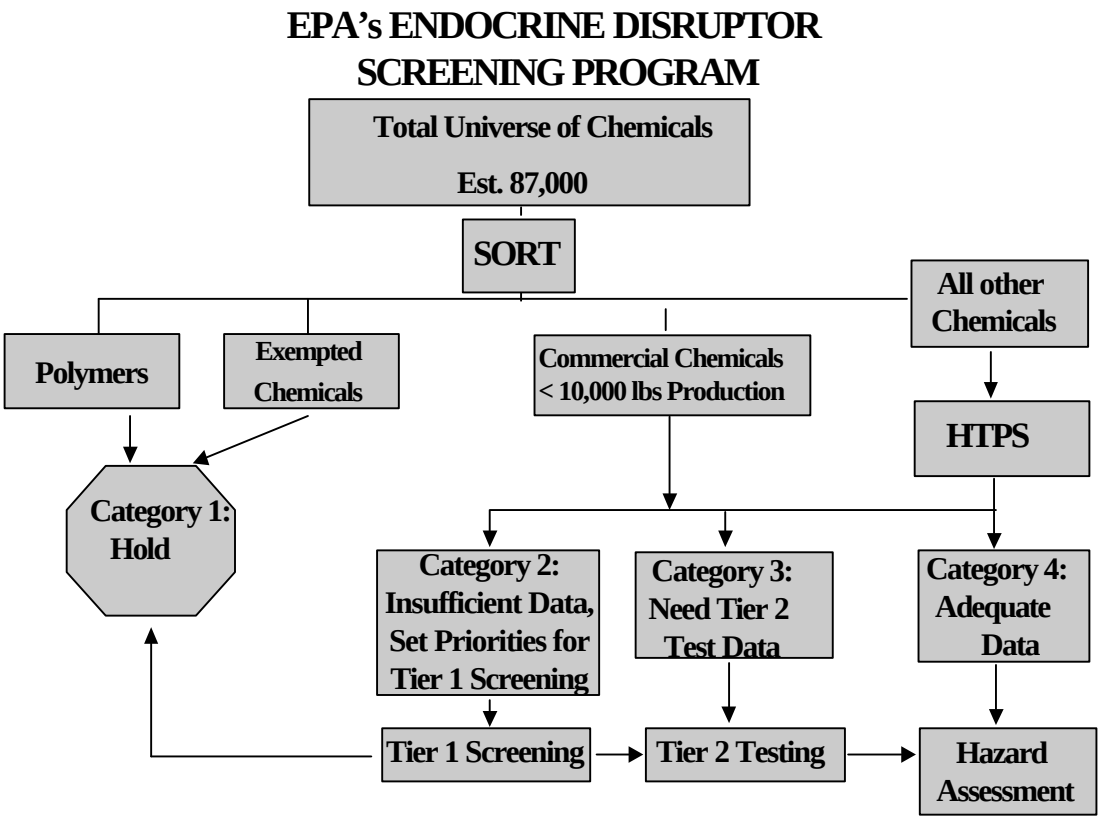
Tier 2 Testing: The purpose of Tier 2 testing is to determine whether an endocrine-active substance causes adverse effects, identify the adverse effects, and establish a quantitative relationship between the dose and the adverse effect. A negative outcome in Tier 2 testing will supersede a positive outcome in Tier 1 screening. Furthermore, each Tier 2 test includes endpoints that will enable EPA to decide whether or not a tested chemical may be considered to be an endocrine disruptor for estrogen, androgen, or thyroid effects.

Once the priorities have been set, the screening will proceed in several phases, beginning with the highest priority chemicals. The phased approach to screening is necessary for practical reasons—the available laboratories and resources for endocrine disruptor screening and testing cannot instantaneously absorb a very large number of chemicals. At the present state of the science, EPA will be able to conclude that a chemical may have an effect similar to a naturally occurring hormone only after it has undergone Tier 2 testing. Therefore, both Tier 1 screening and Tier 2 testing are essential elements of EPA's Endocrine Disruptor Screening Program mandated by the FFDCA.

Moreover, this tiered approach is the most effective strategy for using available resources to detect chemicals that can potentially affect the endocrine system and to quantify those effects. The tiered approach, using a combination of *in vivo* and *in vitro* screens for Tier 1 and *in vivo* tests in Tier 2, has been deemed scientifically reasonable by the NAS (1999) and the Endocrine Disruptor Screening Program Review Subcommittee of the Joint FIFRA Scientific Advisory Panel/US EPA Science Advisory Board (EPA, 1999). For additional details about the tiered approach, please see the proposed statement of policy for the Endocrine Disruptor Screening Program (63 Fed. Reg. 71542; Dec. 28, 1998), available on the EPA web site at *www.epa.gov/fedrgstr/EPA-TOX/1998/December/Day-28/t34298.htm* .

EPA also received public comments on the proposed statement of policy. The Agency will respond to these public comments in a later federal register notice and final statement of policy after it completes scientific investigations regarding the validation and peer review of the screening program's methods.

Figure 1: Flow chart of the Endocrine Disruptor Screening Program



Implementation Progress

The ongoing implementation of EPA's Endocrine Disruptor Screening Program (EDSP) is largely science-driven, based on the recommendations and comments of EDSTAC (1998), the FIFRA SAP/SAB Joint Panel (EPA, 1999), and the NAS (1999). Implementation involves complex and difficult tasks on the cutting-edge of science and science policy. Implementation is currently proceeding on two fronts: priority-setting; and validation of assays. EPA is completing the Endocrine Disruptor Priority Setting Database and the compartment-based approach that the Agency will use to establish priorities for screening chemicals at a later stage of implementation. EPA is also ensuring that the Tier 1 screens and Tier 2 tests[3] that are part of the EDSP are scientifically validated, as required by statute.

Priority Setting

Because pesticide active ingredients generally have more data than other kinds of candidate chemicals, EPA will sort and prioritize pesticide active ingredients separately from the other pesticide formulation ingredients (inerts) and commodity chemicals. For pesticide active ingredients, EPA will review existing reproductive and developmental toxicity data and other relevant scientific information, and sort the chemicals into the four previously described categories.

For pesticide formulation ingredients and commodity chemicals, EPA is completing development of a "compartment-based" approach for evaluating chemicals with little existing data. As mentioned earlier, few data are available on most commodity chemicals, and most of these data are widely disparate. Therefore, direct comparison of Category 2 chemicals with each other is difficult.

To resolve this problem, EPA plans to group chemicals into sets, or compartments. All of the chemicals in a given compartment will have similar kinds of data. Because all chemicals in a compartment will have common information, EPA can compare and prioritize them for screening. Such information might include information pertaining to environmental release, receptor binding, and levels and/or frequency that a chemical has been found in environmental media. For example, the compartment "chemicals in food and drinking water" will rank chemicals on the basis of frequency of occurrence and amounts in which they are found in food and drinking water. EPA will give high priority to chemicals in this compartment if human dietary exposure is expected to be significant.

As part of this compartment-based approach, EPA is also developing a relational data base known as the Endocrine Disruptor Priority Setting Data Base (EDPSD). The EDPSD will contain existing information on chemicals, sort them into compartments, and prioritize them within the compartments. EPA is currently compiling data and information on exposure and effects of chemicals and will complete development of the EDPSD architecture by December 2000. The completion and validation of that part the component of the EDPSD that quantitatively relates a chemical's biological activity with its molecular structure (called

[3]The Tier 1 screens and Tier 2 tests are described in detail in EPA's Endocrine Disruptor Screening Program Proposed Statement of Policy. 63 Fed. Reg. 71,542 (Dec. 28, 1998).

Quantitative Structure-Activity Relationships, or QSAR's), will take an additional year. It is anticipated that the EDPSD will be fully operational by December 2001.

Using High Throughput Pre-screening in Priority Setting

Although the EDSTAC recommended the use of high throughput pre-screening (HTPS) for priority setting, a feasibility study conducted by the Agency demonstrated that the HTPS technology and assay systems were not yet sufficiently developed for routine regulatory application. HTPS was initially viewed as a rapid, efficient means to provide preliminary endocrine effects data. Since all processes are automated and can be programmed to run continuously, large numbers of samples can be screened in a relatively short period of time using this technology. The demonstration of HTPS sponsored by EPA showed that the approach needed more development before routine use. EPA is still considering applying this technology but also has been investigating using QSAR analysis as a feasible and effective alternative.

Using Quantitative Structure-Activity Relationships (QSAR's) in Priority Setting

Quantitative Structure-Activity Relationship (QSAR) analysis represents the use of computer simulations to estimate how a chemical behaves based on its structure. In the case of endocrine disruptors and in the context of the EDSP, the QSAR models would be used to predict the ability of a chemical to bind with estrogen and androgen receptors, usually expressed as binding affinity relative to that of the natural hormone.

Estrogen and androgen receptors are proteins found in cells in many parts of the body. Natural hormones circulating in the body bind with these receptors and may activate or block various biological functions (e.g., growth, sexual development, etc.) associated with the hormone. The process is analogous to a key (the hormone) fitting into a lock (the receptor) and unlocking or locking a door (activation or deactivation of the biological activity). Therefore, chemicals that bind with the receptor may alter or block natural hormone function, and such alteration or blocking is the first step in causing certain types of effects.

Since QSAR's predict a chemical's propensity to bind with a hormone receptor, QSAR analysis provides EPA with only limited information on the potential for a chemical to interfere with the endocrine system. As explained in the Overview, receptor binding affinity is only one of various ways that a chemical can affect the endocrine system. EPA is considering several different QSAR approaches to assist the Agency in setting priorities in its screening program. EPA will perform the QSAR analysis on most pesticide active ingredients, other pesticide formulation ingredients, and selected commodity chemicals.

Scientific Screen and Test Validation

Scientific screen and test validation consists of developing a method for conducting a specific scientific screen or test, evaluating the screen/test's ability to achieve its stated purpose, and ensuring that it can be performed reliably and consistently in different laboratories. At the present time, there are no adequately validated screens for determining if a substance may have an effect in humans that is similar to an effect produced by a naturally occurring hormone. Validation is a time and resource intensive activity.

For endocrine disruptors, the specific screens and tests being validated are the Tier 1 screens and Tier 2 tests specified in EPA's Endocrine Disruptor Screening Program Proposed Statement of Policy. The EDSP screen/test validation process consists of several general stages: 1) screen/test development; 2) demonstration that the screen/test achieves its purpose and is reliable, leading to a standardized protocol[4] ; 3) a study to determine if the standardized protocol can be performed consistently at different laboratories with comparable results; and 4) independent scientific peer review of the study results.

EPA currently is working on standardizing and validating the following Tier 1 screens: a uterotrophic screen; a Hershberger screen; a rodent pubertal female screen; a rodent pubertal male screen; estrogen and androgen receptor reporter gene screens; a fish reproduction screen; and a frog metamorphosis screen. EPA is also actively working toward validating the following Tier 2 tests: a two generation mammalian reproduction and development test and a mysid shrimp reproduction and development test. In addition, EPA has convened and participated in meetings and workshops regarding the development of initial protocols for a mammalian developmental screening test and an avian reproduction test.

Because certain of the proposed EDSP screens and tests also are of international interest, EPA is working with the Organisation for Economic Co-operation and Development (OECD) to standardize and validate several of the tests included in the program. All of the EPA validation work is being conducted in close liaison with the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) established by the National Toxicology Program, under the auspices of the National Institute of Environmental Health Sciences (NIEHS), and is following the ICCVAM principles.

Following are brief discussions pertaining to each of the screens and tests currently being validated:

The Uterotrophic Screen. The rodent uterotrophic screen is designed to screen chemicals for estrogenic activity. It detects the ability of a chemical to stimulate or inhibit estrogenic responses of the uterus. The OECD, with participation from EPA, has developed the screen and standardized the protocol. Preliminary findings suggest that the screen is robust and ready for inter-laboratory validation.

The Hershberger Screen. The Hershberger screen is designed to screen chemicals for androgenic activity. It detects the ability of a chemical to stimulate or inhibit androgenic responses in the testes and secondary sex organs. The OECD, with participation from EPA, has completed the development of the rodent Hershberger protocol, and has initiated a study to standardize and validate the protocol. EPA scientists are leading the coordination of this effort.

The Rodent Pubertal Female Screen. The rodent pubertal female screen is designed to screen for estrogenic and thyroid activity in immature female animals exposed to chemicals as the animals undergo sexual maturation. The screen examines abnormalities associated with development of the female sex organs and secondary sexual characteristics. The Agency is in the process of standardizing the operational protocol for the rodent pubertal female screen. An EPA

[4] A protocol contains the detailed, step-by-step instructions for conducting a screen/test.

contractor has conducted this demonstration using six endocrine active chemicals and two different strains of laboratory rats.

The Rodent Pubertal Male Screen. The rodent pubertal male screen is designed to screen for androgenic and thyroid activity in immature male animals exposed to chemicals as they undergo sexual maturation. The screen examines abnormalities associated with development of the male sex organs and secondary sexual characteristics. The Agency is in the process of standardizing the operational protocol for the rodent pubertal male screen. An EPA contractor has conducted this demonstration using six endocrine active chemicals and two different strains of laboratory rats.

Fish Reproduction Screen. The fish reproduction screen is designed to screen chemicals for estrogenic and androgenic effects. The method examines abnormalities associated with survival, reproductive behavior, secondary sex characteristics, and fecundity (number of spawns, number of eggs per spawn, fertility, and development of offspring). The fish reproduction screen has undergone development and demonstration of an operational protocol. EPA has conducted the demonstration with four endocrine active chemicals and prepared a report of the study results.

The Frog Metamorphosis Screen. The frog metamorphosis screen is designed to screen chemicals for thyroid effects. Metamorphosis is under thyroid control and is a surrogate for screening potential thyroid effects in humans. The screen examines abnormalities associated with the tail resorption of tadpoles as they metamorphose into frogs. The frog metamorphosis screen has undergone screen development and demonstration of an operational protocol. EPA has awarded a contract to conduct the demonstration with four endocrine active chemicals, has received the data, and has prepared an initial draft report of the results.

Estrogen and Androgen Receptor Reporter Gene Screens and Other *In Vitro* Screens. Estrogen and Androgen Receptor Reporter Gene screens are designed to detect the consequences of binding to the estrogen or androgen receptor (activation or deactivation of a biological process or blocking of the receptor). Reporter gene screens provide actual measurements, as compared with computer simulated estimates provided by the QSAR analyses described earlier. EPA has initiated methods development research on both estrogen receptor and androgen receptor reporter gene screens using mammalian, fish, and amphibian tissue/systems, a DNA membrane method for screening thyroid activity, and has initiated a review of DNA array technology to evaluate its potential for use in endocrine disruptor screening.

Mysid Shrimp (Invertebrate) Reproduction Test. The mysid shrimp test is designed to characterize the dose-response characteristics and adverse reproductive and developmental effects of chemicals in this invertebrate species. EPA has developed and successfully demonstrated the use of a protocol for a two-generation Tier 2 test for the mysid shrimp.

Mammalian 2-Generation Reproduction Test. The EPA/OPPTS revised Test Guideline on Reproduction and Fertility Effects (OPPTS 870.3800, August 1998) is designed to characterize dose-response characteristics and adverse reproductive and developmental effects of chemicals in mammals and is used to evaluate the potential for impact on both humans and other mammalian species. The OPPTS 870.3800 test guideline contains new and important endpoints for estrogenic and androgenic effects. In addition, EPA is currently updating the guideline to add

important endpoints for evaluating thyroid effects. This new protocol is currently being demonstrated.

Table 1: Estimated Completion Dates for Validation of the EDSP Screens and Tests.

Tier 1 Screen/Test	Pre-validation	Validation
ER/AR Binding	2000	2001
Steroidogenesis	2001	2002
Aromatase	2001	2002
Uterotrophic	2000	2001
Hershberger	2000	2001
Pubertal Female	2001	2002
Pubertal Male	2001	2002
In utero/lactation	2001	2003
Frog Thyroid	2001	2002
Fish Reproduction Screen	2001	2002
Tier 2 Test		
Mammalian 2-gen	2001	2003
Avian	2002	2003
Fish	2001-2002	2004
Amphibian	2002-2003	2005
Invertebrate	2003-2004	2004

Regulatory Implementation

Prior to regulatory implementation, EPA will publish final test guidelines within six to twelve months after the EDSP screens and tests have been validated. In addition, the Agency will publish a final statement of policy and an accompanying federal register notice which will set forth the final policy and procedures for implementation of the EDSP. At that time, EPA will fully address comments submitted in response to the Proposed Statement of Policy published on December 28, 1998.

EPA will not require that all chemicals be screened at once. Because of the large number of chemicals and new protocols, EPA proposes requiring screening in phases, starting with the highest-priority chemicals. This will allow chemicals perceived to pose the highest potential risk to be tested first, and will allow testing facilities and industry to build capacity for conducting the screens and tests.

Once the screens and tests for health effects are available, EPA plans to implement screening and testing requirements in several phases. Phase I would involve pesticide active ingredients and other pesticide formulation ingredients with high production volume. Subsequent phases would likely address commercial chemicals and environmental contaminants.

EPA anticipates issuing orders under FFDCA section 408(p) and/or data call ins under FIFRA 3(c)(2)(B) to require Tier 1 screening of pesticide active ingredients and other pesticide formulation ingredients with high production volume in 2003. Orders could be issued as early as 2004 for Tier 2 testing for agricultural (i.e. food-use) pesticides and a few other chemicals. EPA will publish a proposed list of chemicals for Phase I screening approximately one year in advance of the date screening is required to begin. The Agency will invite chemical producers, pesticide registrants and others, including the public, to submit data on the listed chemicals if, in their judgment, this information might materially affect a chemical's status on the list. The data may include information about physical/chemical characteristics, exposure, human health effects, or environmental effects. Such data may raise or lower a chemical's priority for Tier 1 screening or may shift it from the screening priority list to the Tier 2 testing list.

Endocrine Disruptor Research

To evaluate fully the potential risk to humans and wildlife of exposure to endocrine disruptors in the environment, EPA intends to collect data beyond those developed through its screening program. Uncertainties regarding abnormal development and reproduction in human and wildlife populations, neurological effects, immunologic effects, carcinogenic effects, and other effects of wildlife have been identified as areas of research by EPA (1997, 1998) and the National Academy of Sciences (NAS, 1999).

The NAS recommendations are consistent with the research gaps identified by the Committee on Environment and Natural Resources (CENR) of the National Science and Technology Council. Much of the recommended research and monitoring efforts are either underway or planned by federal agencies as part of the federal Endocrine Disruptor Research Initiative developed using the CENR framework. EPA and other federal agencies, under the

auspices of the CENR, have awarded grants for research on population-level effects in wildlife and effects on human health, and are proposing to make awards in response to a joint Request for Applications (RFA) for grants for studies that will investigate the relationships between exposure to endocrine disruptors and reproductive/developmental effects in humans. EPA resources, leveraged with those of other federal agencies, will be used to determine, among other effects: 1) whether or not there is reduced fertility in exposed human males and females; 2) pregnancy outcomes of exposed human females; and 3) the incidences of hormonally mediated cancers of the reproductive tract in human male and female offspring exposed *in utero*.

Alternative Test Method Development

EPA is committed to minimizing the number of animals that will be used in implementing the EDSP, and to incorporating alternative test methods whenever and wherever possible. The goal of the EDSP is to predict the potential of chemicals to disrupt the endocrine system of humans and wildlife, including threatened and endangered species. EPA proposes to do this using a combination of *in vitro* and *in vivo* systems, using laboratory animals as models, or surrogates. Humans and higher-order animals have complex organ systems, mechanisms for distributing chemicals in the body, and metabolic processes for isolating, counteracting, and removing toxicants. At this time, only whole animal testing allows scientists to observe the interactions that are occurring in humans and higher-order animals with respect to their biochemical processes and organ systems. Therefore, other mammalian species such as rats and mice are utilized to provide an understanding of the complexity of the human and higher-order animal body response when exposed to a test substance.

New and revised toxicological test methods, however, are being developed with increasing frequency, and scientists around the world are incorporating recent advances in molecular and cellular biology, as well as new research technologies, into their work. These developments hold great promise for reducing animal use in testing in the future.

As co-chair of the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM), EPA is working to ensure that alternative tests are validated and accepted, and will endeavor to incorporate those alternatives into its testing programs as quickly as possible. ICCVAM is a standing committee consisting of 14 federal agencies. It coordinates validation, acceptance, and national/international harmonization of toxicological test methods. EPA has allocated funds in FY00 and FY01 to support alternative test method development.

Findings

1. The EDSP should identify and characterize effects related not only to estrogen, but to androgen and thyroid hormones, as well.
2. There currently are no adequately validated routine screens or tests for determining whether a substance may produce an effect in humans similar to an effect produced by a naturally occurring estrogen or any other naturally occurring hormone. EPA is in the process of standardizing and validating screens and tests for use under the EDSP.

3. The EDSTAC recommended that the EDSP address effects on both humans and fish and wildlife.
4. Sorting and priority setting tools and processes are needed and are being developed.
5. EPA has conducted a feasibility study of the high throughput pre-screening (HTPS) process suggested by the Endocrine Disruptor Screening and Testing Advisory Committee, which showed that the approach needed more development before routine use. At this time, it appears that similar information can be more efficiently derived from other non-animal methods such as the Quantitative Structure Activity Relationship (QSAR) computer simulation model.
6. Research and environmental monitoring programs are needed to characterize more fully the nature and magnitude of environmental exposures for risk assessment purposes.

Recommendations for Further Testing

At this point in the implementation of the program, EPA has no specific recommendations for testing in addition to the testing that it plans to require in later stages of implementation. The Agency encourages and supports the development of new techniques that will allow the Endocrine Disruptor Screening Program to proceed in ways that enhance the program's scientific integrity and capabilities to predict the potential for substances to cause endocrine disruption, while minimizing animal usage.

Recommendations for Further Actions

Recommendation: Continue to implement the EDSP initial sorting, priority setting, and screening and test method standardization and validation efforts.

Recommendation: Continue to support endocrine disruptor research and monitoring efforts recommended by the National Academy of Sciences (NAS) and the Committee on Environment and Natural Resources (CENR) of the National Science and Technology Council.

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