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Part II

Environmental Protection Agency

Endocrine Disruptor Screening Program: Statement of Policy; Notice

Endocrine Disruptor Screening Program: Priority-Setting Workshop; Notice

ENVIRONMENTAL PROTECTION AGENCY

[OPPTS–42208; FRL–6052–9]

Endocrine Disruptor Screening Program; Proposed Statement of Policy

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice.

SUMMARY: In this notice, EPA is providing additional details and an opportunity for public comment on its Endocrine Disruptor Screening Program (EDSP). The Agency first set forth the basic components of the EDSP in the August 11, 1998, **Federal Register**. The EDSP is required by the Federal Food, Drug, and Cosmetics Act (FFDCA), as amended by the Food Quality Protection Act (FQPA). In developing the EDSP, EPA considered recommendations of the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), a panel chartered pursuant to the Federal Advisory Committee Act. EDSTAC recommended expansion of the screening program beyond the statutory minimum to include not only pesticides but commercial chemicals regulated under the Toxic Substances Control Act (TSCA), certain natural products, non-pesticide food additives, and cosmetics. EDSTAC also recommended that EPA screen for effects on the androgen and thyroid systems and for effects on fish and wildlife. This notice describes the major elements of EPA's EDSP, as well as its implementation. EPA is seeking public comment on the EDSP in this notice.

DATES: Written comments on this proposed policy must be received by EPA on or before February 26, 1999.

The joint meeting of the EPA Science Advisory Board (SAB) and Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) to review EPA's proposal for the EDSP will be held March 30 through April 1, 1999. A document announcing the meeting sites and times will be published in the **Federal Register**.

ADDRESSES: Each comment must bear the docket control number OPPTS–42208. All comments should be sent in triplicate to: OPPT Document Control Officer (7407), Office of Pollution Prevention and Toxics, Environmental Protection Agency, 401 M St., SW., Room G–099, East Tower, Washington, DC 20460.

Comments and data may also be submitted electronically to: oppt.ncic@epa.gov. Follow the instructions under Unit IX. of this notice. No Confidential Business Information (CBI) should be submitted through e-mail.

All comments which contain information claimed as CBI must be clearly marked as such. Three sanitized copies of any comments containing information claimed as CBI must also be submitted and will be placed in the public record for this rulemaking. Persons submitting information on any portion of which they believe is entitled to treatment as CBI by EPA must assert a business confidentiality claim in accordance with 40 CFR 2.203(b) for each such portion. This claim must be made at the time that the information is submitted to EPA. If a submitter does not assert a confidentiality claim at the time of submission, EPA will consider this as a waiver of any confidentiality claim and the information may be made available to the public by EPA without further notice to the submitter.

FOR FURTHER INFORMATION CONTACT: For general information or copies of the EDSTAC Final Report: TSCA Hotline, Environmental Assistance Division (7408), Office of Pollution Prevention and Toxics, Environmental Protection Agency, 401 M St., SW., Washington, DC 20460; telephone (202) 554–1404, TDD (202) 554–0551; e-mail address: TSCA-Hotline@epa.gov. For technical information, please contact Anthony Maciorowski, Office of Pesticide Programs, telephone: (202) 260–3048, e-mail address: maciorowski.anthony@epa.gov or Gary Timm, Chemical Control Division, Office of Pollution Prevention and Toxics, telephone: (202) 260–1859, e-mail address: timm.gary@epa.gov.

SUPPLEMENTARY INFORMATION

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I. General Information

A. Does this notice apply to me?

This notice describes the major elements of EPA's EDSP, and also requests public comments on technical and policy aspects of the program. You may be interested in the program set forth in this notice if you produce, manufacture or import pesticide chemicals, chemical substances or mixtures subject to TSCA, substances that may have an effect cumulative to an effect of a pesticide, or substances found in sources of drinking water. The general public may also have an interest in the potential health and environmental consequences associated with the results of any testing that is conducted in conformity with this policy. If you have any questions regarding the applicability of this action to a particular entity, consult the technical person listed under ''FOR FURTHER INFORMATION CONTACT.''

B. How can I get additional information or copies of this notice or other support documents?

1. *Electronically*. You may obtain electronic copies of this notice and various support documents from the EPA Home Page at http://www.epa.gov/. On the EPA Home Page select ''Laws

and Regulations'' and then look up the entry for this notice under ''**Federal Register**—Environmental Documents.'' You can also go directly to the ''**Federal Register**'' listings at http://www.epa.gov/fedrgstr/.

The complete EDSTAC Final Report is available on the worldwide web at: www.epa.gov/opptintr/opptendo/whatsnew.htm. Paper copies of the EDSTAC Final Report can be obtained upon request from the TSCA Hotline at the address listed under ''FOR FURTHER INFORMATION CONTACT'' section of this notice.

2. *In person or by phone.* If you have any questions or need additional information about this action, please contact the technical person identified under ''FOR FURTHER INFORMATION CONTACT.'' A public version of this record, including printed, paper versions which does not include any information claimed as CBI, is available for inspection in the TSCA Nonconfidential Information Center, Rm. NE–B607, 401 M St., SW., Washington, DC, 12 noon to 4 p.m., Monday through Friday, excluding legal holidays. The telephone number of the TSCA Docket is (202) 260–7099.

II. Background

A. Concern Regarding Endocrine Disruptors

The endocrine system consists of glands and hormones which are found in all mammals, birds, fish, and invertebrates. Hormones are biochemical substances produced in glands and released into the blood stream to act on an organ in another part of the body. Over 50 hormones have been identified in humans and other vertebrates. Hormones control or regulate many biological processes and are often produced in exceptionally low amounts within the body. Examples of such processes include blood sugar control (insulin); differentiation, growth, and function of reproductive organs (testosterone (T) and estradiol); and body growth and energy production (growth hormone and thyroid hormone). Much like a lock and key, many hormones act by binding to receptors that are produced within cells. The hormone-receptor complex switches on or switches off specific biological processes in cells, tissues, and organs.

Scientific evidence has been accumulating that humans, domestic animals, and fish and wildlife species have exhibited adverse health consequences from exposure to environmental chemicals that interact with the endocrine system. To date, such problems have been detected in domestic or wildlife species with relatively high exposure to organochlorine compounds (e.g., 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane (DDT) and its metabolite dichorodiphenyldichloroethylene (DDE), polychlorinated biphenyls (PCBs), and dioxins) or to some naturally occurring plant estrogens. But effects from exposure to low levels of endocrine disruptors has been observed as well (e.g., parts per trillion levels of tributyl tin have caused masculinization of female marine molluscs such as the dog whelk and ivory shell). Adverse effects have been reported for humans exposed to relatively high concentrations of certain contaminants. However, whether such effects are occurring in the human population at-large at concentrations present in the ambient environment, drinking water, and food remains unclear. Several conflicting reports have been published concerning declines in the quality and quantity of sperm production in humans over the last 4 decades, and there are reported increases in certain cancers (e.g., breast, prostate, testicular). Such effects may have an endocrine-related basis, which has led to speculation about the possibility that these endocrine effects may have environmental causes. However, considerable scientific uncertainty remains regarding the actual causes of such effects. Nevertheless, there is little doubt that small disturbances in endocrine function, particularly during certain highly sensitive stages of the life cycle (e.g., development, pregnancy, lactation) can lead to profound and lasting effects (Kavlock et al., 1996. EPA, 1997).

Taken collectively, the body of scientific research on human epidemiology, laboratory animals, and fish and wildlife provides a plausible scientific hypothesis that environmental contaminants can disrupt the endocrine system leading to adverse-health consequences. A critical issue is whether ambient environmental levels are sufficiently high to exert adverse effects on the general population. Various types of scientific studies (epidemiology, mammalian toxicology, and ecological toxicology) are necessary to resolve many of the scientific questions and uncertainty surrounding the endocrine disruptor issue. Many such studies are currently underway by government agencies, industry, and academia.

B. The Food Quality Protection Act, Safe Drinking Water Act, and Other Environmental Legislation

In 1996, Congress amended the FFDCA with the FQPA. FFDCA section 408(p) requires EPA to develop a program ''to determine whether certain substances may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or such other endocrine effects as [EPA] may designate'' (FFDCA section 408(p) (21 U.S.C. 346a(p))).

When carrying out the program, EPA ''shall provide for the testing of all pesticide chemicals'' and ''may provide for the testing of any other substance that may have an effect that is cumulative to an effect of a pesticide chemical if the Administrator determines that a substantial population may be exposed to such a substance'' (21 U.S.C. 346a(p)(3)).

In addition, Congress amended the Safe Drinking Water Act (SDWA) and gave EPA authority to provide for the testing, under the FQPA 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'' (SDWA Amendments of 1996, section 136 (42 U.S.C. 300j–17)).

This notice describes the major elements of the program EPA has developed to comply with the requirements of FFDCA section 408 (p) as amended by FQPA. EPA initially set forth the Program in an August 11, 1998, **Federal Register** notice (63 FR 42852) (FRL–6021–3). The screening program described in this notice is ambitious. EPA is considering 87,000 substances as potential candidates for testing. EPA believes that the FFDCA and SDWA provide authority to require the testing of many of these substances. EPA will use other testing authorities under the FIFRA and TSCA to require the testing of those chemical substances that the FFDCA and SDWA do not cover. EPA also plans to work with other Federal agencies and departments to ensure that substances not covered under any of EPA's authorities are tested.

As described in detail in this unit, the EDSP is divided into several stages, including a priority-setting stage, a stage involving screening tests (Tier 1 screening), and a stage involving confirmatory testing (Tier 2 testing). EPA believes that the results from the entire battery of tests required in the Tier 1 screening and Tier 2 testing stages (or their equivalents) are necessary to make the statutory determination of whether a particular

substance ''may have an effect in humans that is similar to an effect produced by a naturally occurring [hormone]''(21 U.S.C. 346a(p)). In other words, a positive result in the Tier 1 screening assays would not be adequate to make the determination ''whether a substance may have an effect in humans that is similar to an effect produced by a naturally occurring [hormone].'' Id. Conversely, a negative result in all Tier 1 screening tests will be adequate to determine that a particular substance is not likely to have an effect on the estrogen, androgen, and thyroid hormone systems (EAT) and, therefore, is not a priority for testing in Tier 2. The confirmatory tests in the Tier 2 testing stage are necessary to determine whether a substance may have an effect similar to that of a naturally occurring hormone.

C. The 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 it on developing a program to comply with FFDCA section 408(p) requirements. The Advisory Committee, known as the EDSTAC, was comprised of members representing the commercial chemical and pesticides 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 charged EDSTAC with developing the following:

Methods for chemical selection and priorities for screening.

1. A set of available, validated screening tests for early application.

2. Ways to identify new and existing screening tests and mechanisms for their validation.

3. Processes and criteria for deciding when additional tests beyond screening would be needed and how to validate such tests.

4. Processes for communicating to the public about the EDSTAC's agreements, recommendations, and information developed during priority setting, screening, and testing.

In response to this charge, EDSTAC reached consensus on a set of recommendations for the Agency. These recommendations are contained in the EDSTAC Final Report (EDSTAC, 1998). Considering EDSTAC's diverse membership—including individuals from industry, labor, environmental justice groups, public health and environmental groups, academia, and Federal and State agencies—EPA found its consensus 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 its EDSP. EPA has not further developed recommendations in areas where EDSTAC recommended further stakeholder involvement. However, in other areas, EPA has added additional refinements which are highlighted under ''Issues for Comment'' in Unit VII. of this notice.

D. Key Terms and Definitions

For the purposes of this notice, EPA will use the following definitions.

Chemical or chemical substance as used in this notice includes naturally occurring and synthetic chemicals and elements.

Commercial chemical is defined as chemical substances subject to the provisions of TSCA (15 U.S.C. 2602 *et seq.*).

Exempted chemicals are pesticide chemicals that have been given an exemption under FFDCA section 408(p) or commercial chemicals that the Agency determines to exempt from the requirements of screening and are therefore not subject to the EDSP.

Functional equivalency—an assay, test, or endpoint may be defined as being ''functionally equivalent'' to another assay, test, or endpoint when it provides equivalent information for each endpoint being studied. For purposes of the EDSP, assays, tests, and endpoints must be standardized and validated prior to use. The standardization and validation process will provide data and information that will allow EPA to develop guidance on the use of functionally equivalent assays, tests, and endpoints prior to the implementation of the screening program.

Hazard assessment is defined to include identification of the chemical substances and mixtures that have endocrine-disruption effects (which is often referred to as hazard identification) and establishment of the relationship between dose and effect (which is often referred to as dose-response assessment).

Mixtures refers to combinations of two or more chemical substances, including those found in the environment. This definition is the ordinary definition applied by chemists and differs from the legal definition under TSCA section 3. The TSCA definition of mixture excludes natural products and chemical reaction products that may be a combination of two or more chemical substances.

Pesticide chemical means any substance that is a pesticide within the meaning of FIFRA, including all active and inert ingredients of such pesticide and all impurities.

Polymer is defined as a chemical substance consisting of one or more types of monomer units and comprising a simple weight majority of molecules containing at least three monomer units which are covalently bound to at least one other monomer unit or other reactant and which consists of less than a simple weight majority of molecules of the same molecular weight. Such molecules must be distributed over a range of molecular weights wherein differences in the molecular weight are primarily attributable to differences in the number of monomer units.

Priority setting is defined as the collection, evaluation, and analysis of relevant information, including the results of HTPS, to determine the general order in which chemical substances or mixtures will be subjected to screening and testing.

Screening is defined as the application of short-term assays to determine whether a chemical substance or mixture may interact with the endocrine system. As these are preliminary assays, a positive result during screening does not mean that a chemical substance may have an effect in humans, fish, or wildlife that is similar to the effect produced by naturally occurring hormones.

Sorting is the separation of chemicals into groups prior to priority setting for the purpose of distinguishing chemicals needing Tier 1 screening from those needing Tier 2 testing, hazard assessment, and those for which endocrine screening, testing, or hazard assessment is not warranted at this time.

Testing is defined as a customized combination of long-term assays and endpoints designed to determine whether a chemical substance or mixture may cause effects in humans, fish, or wildlife that are similar to effects caused by naturally occurring hormones and to identify, characterize, and quantify these effects. Tests are designed to confirm and further define the results obtained in Tier 1 screens.

Weight-of-evidence refers to the process by which trained professionals judge the strengths and weaknesses of a collection of information to render an

overall conclusion that may not be evident from consideration of the individual data.

III. Overview of the Screening Program

A. Scope

Based on the body of available scientific information, EDSTAC recommended that EPA's EDSP address both human and ecological (fish and wildlife) effects; examine effects to EAT-related processes; and include chemical substances and representative mixtures. EPA fully agrees with the EDSTAC that this is the appropriate scope for the initial EDSP.

For the reasons stated in this unit, EPA is proposing that the EDSP include the following:

1. *Human and ecological (fish and wildlife) effects.* 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.

2. *Effects on EAT-related processes.* Initially, the EDSP will focus on EAT effects. These three hormone systems are presently among the most studied of the approximately 50 known vertebrate hormones. *In vitro* and *in vivo* test systems to examine EAT effects exist, and are currently the most amenable for regulatory testing. Further, inclusion of 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.

3. *Evaluate endocrine disrupting properties of chemical substances and common mixtures.* The universe of chemicals and mixtures to be prioritized for endocrine-disruptor screening and testing numbers more than 87,000 and includes commercial chemicals, active pesticide ingredients, ingredients in cosmetics, nutritional supplements, and food additives. Commercial chemicals are being included because chemicals like PCBs and other non-pesticidal chemicals have been implicated as endocrine disruptors. Nutritional supplements are known to contain certain naturally occuring phytoestrogens. In addition, EPA plans to screen representative examples of six different types of mixtures (i.e., combinations of two or more chemicals). The inclusion of the representative mixtures was viewed to be a pragmatic, achievable first look at a highly complex problem. Testing mixtures will determine whether mixtures cause different endocrine effects from those of the individual component chemicals. While pharmaceuticals will not be tested per se since they are already tested and highly regulated for human or animal use, they may be tested as pollutants if found to be present in the environment.

B. Program Elements

EPA will use a tiered approach for determining whether a substance may have an effect in humans that is similar to an effect produced by naturally occurring EAT. The core elements of the tiered approach include: Sorting, priority setting, Tier 1 screening, and Tier 2 testing. The purpose of Tier 1 is to identify substances that have the potential to interact with the endocrine system. The purpose of Tier 2 is to determine whether the substance causes adverse effects, identify the adverse effects caused by the substance, and establish a quantitative relationship between the dose and the adverse effect. At this stage of the science, only after completion of Tier 2 tests will EPA be able to determine whether a particular substance may have an effect in humans that is similar to an effect produced by a naturally occurring EAT, that is, that the substance is an endocrine disruptor. Therefore, both Tier 1 and Tier 2 are essential elements of the screening program mandated by the FQPA. Moreover, this tiered approach is the most effective strategy for using available resources to detect endocrine-disrupting chemicals and quantify their effects. The core elements of the program are introduced in this overview section and presented in greater detail in subsequent sections.

Some of the major implementation steps and estimated completion dates are:

Implementation steps	Estimated completion dates
EDSTAC Final Report and Recommendations	Completed
Development of EPA's EDSP	Completed
Public comment on EPA's EDSP	February 22, 1999
SAB/SAP Peer Review Processes	April 1, 1999
HTPS Demonstration	February 1999
HTPS	June 2000
EDPSD	June 2000
Priority Setting for Tier 1 Phase 1	November 2000
Tier 1 Standardization and Validation September	2001
Tier 1, Phase 1 TSCA Test Rule Notice of Proposed Rulemaking (NPRM) and FQPA Orders	December 2001
Tier 1, Phase 1 TSCA Final Test Rule	June 2003

IV. Sorting and Priority Setting

A. The Universe of Chemicals Included in the EDSP

As stated earlier, EPA is concerned about the endocrine disrupting potential of more than 87,000 chemical substances, including pesticide chemicals, commercial chemicals, ingredients in cosmetics, food additives, nutritional supplements, and certain mixtures. Testing of all of these chemicals cannot be supported at the same time because, even if EPA and industry had the resources to do so, there are not enough laboratories or other facilities capable of conducting the testing. Consequently, EPA has included a priority-setting phase as part of its EDSP. During the priority-setting phase, EPA will use existing information, and in some cases, preliminary test results, to prioritize chemicals for testing. While EPA believes that the FFDCA and SDWA provide authority to require the testing of many of these substances, EPA also will use other testing authorities under FIFRA and TSCA to require the testing of those chemical substances that the FFDCA and SDWA do not cover. EPA also plans to work with other Federal agencies and departments to ensure that these substances also are tested. EPA will use appropriate authority to obtain testing of the chemical.

B. Sorting

Chemicals under consideration for EAT screening will undergo sorting based on existing, scientifically relevant information. The sort would identify chemicals for HTPS as well as place chemicals into categories 1–4.

1. *Category 1—Hold—Chemicals with sufficient, scientifically relevant information to determine that they are not likely to interact with the EAT.* If

EPA is able to determine, based on scientifically relevant information, that a specific chemical is not likely to interact with the EAT, it will place that chemical in a hold category. Chemicals in this hold category will have the lowest priority for further analysis and may not undergo further analysis unless new and compelling information suggests that the chemical may interact with the endocrine system. Although EPA will place chemicals in the hold category during the initial sorting phase of the screening program, it may add chemicals to this category if, during a later phase of the EDSP (Tier 1 screening, or Tier 2 testing), the Agency determines that a particular chemical is not likely to interact with the endocrine system.

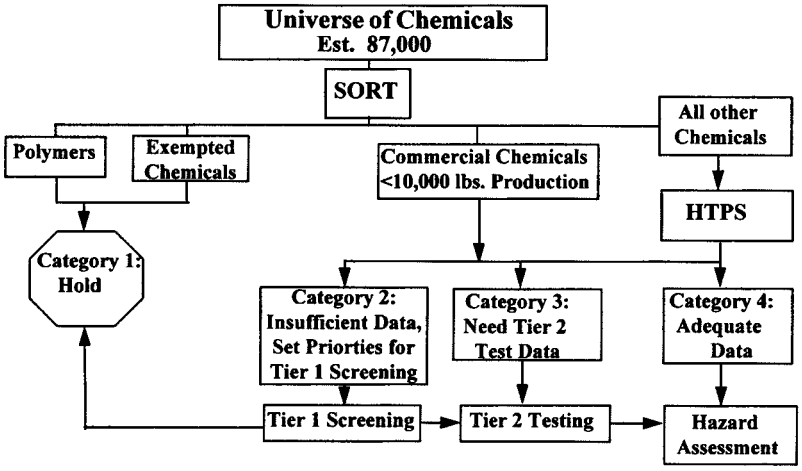
Currently, EPA believes it is appropriate to assign two groups of chemicals to the hold category:

i. Polymers.

ii. Exempted chemicals.

These substances would not be subject to HTPS or to priority setting for screening at this time (See Fig. 1).

FIGURE 1-SCREENING PROGRAM OVERVIEW



i. *Polymers*. EPA anticipates placing most polymers with a number average molecular weight (NAMW) greater than 1,000 daltons in the hold category. These polymers are not likely to cross biological membranes and therefore are not likely to be biologically available to cause endocrine-mediated effects. EPA will not place polymers that are pesticide chemicals, and therefore must be tested under the FFDCA, in this category. In addition, EPA will not place monomer and oligomer components of polymers in this hold category. Instead, it will prioritize them for Tier 1 screening or Tier 2 testing.

ii. *Exempted chemicals*. Exempted chemicals are pesticides given an exemption under FFDCA 408(p) and other chemicals that the Agency determines to exempt from the requirements of screening. These substances would not be included in the HTPS and would be placed in the hold category (see Unit. VI.L. of this notice).

2. *Category 2—Priority Setting/Tier 1 Screening—Chemicals for which there is insufficient, scientifically relevant information to determine whether or not they are likely to interact with the EAT*. If EPA is not able to determine, based on scientifically relevant information, whether or not a chemical is likely to interact with the EAT, it will place that chemical into a category of chemicals needing Tier 1 screening. Category 2 chemicals are those for which there is insufficient scientifically relevant information to be placed on hold (Category 1), or assigned to Tier 2 testing (Category 3) or hazard assessment (Category 4). Category 2 chemicals will be subjected to formal priority setting, and Tier 1 screening, and as appropriate (i.e. positive results in Tier 1 screening), Tier 2 testing.

3. *Category 3—Tier 2 Testing—Chemicals with sufficient, scientifically relevant information comparable to that provided by the Tier 1 screening*. Recognizing the need for flexibility, EPA has included the possibility of bypassing Tier 1 screening. For example, if sufficient, scientifically relevant information already exists regarding a specific chemical, EPA may move that chemical directly into Tier 2 testing. In addition, EPA may allow a chemical to bypass Tier 1 if the chemical's producer or registrant chooses to conduct Tier 2 testing without performing Tier 1 screening.

4. *Category 4—Hazard Assessment—Chemicals with sufficient, scientifically relevant information to bypass Tier 1 screening and Tier 2 testing*. For certain chemicals, there already may be sufficient, scientifically relevant information regarding their interaction with EAT—information comparable to that derived from Tier 1 screening and Tier 2 testing—to move them directly into hazard assessment. These chemicals, thus, will bypass both Tier 1 screening and Tier 2 testing. EPA anticipates that this will be a relatively small number of chemicals.

C. Information Required for Sorting and Priority Setting

Relevant scientific information is essential to sort and prioritize chemicals for endocrine-disruptor testing. EPA plans to use three main categories of information to set priorities: Exposure-related information, effects-related information, and statutory criteria. EPA is in the process of developing a relational data base to manage the information that it will use to set priorities. A relational data base is one that can link with other data bases thus allowing EPA to access and manipulate data from other existing data bases.

1. *Exposure-related information and criteria*. EPA proposes to use several types of existing exposure-related information and criteria for initial sorting and priority setting. These include at least four exposure information categories and one fate and transport information category. The four exposure-related information categories are: Biological sampling data for humans and other biota; environmental monitoring data, and information on occupational , consumer product, and food-related exposures; data on environmental releases; and data on production volume and use. Note that the data categories are listed from most robust (actual presence in biological tissue confirming that exposure has occurred) to least robust (amounts produced which may or may not result in exposure).

This unit describes the nature of the information included in each exposure-related information category, the strengths and limitations of the type of information in each category, and a set of guiding principles that EPA will generally apply to complete the task of setting priorities for endocrine-disruptor screening and testing.

i. *Biological sampling data*. Biological sampling refers to the monitoring of tissues from live or dead organisms for chemicals to document actual human or animal exposure. Biological sampling information falls into two subcategories: Human biomonitoring and monitoring of other biota. Human biomonitoring includes human tissues and media (e.g., blood, breast milk, adipose tissue, and urine). Monitoring of other biota encompasses a wide range of species (invertebrates, vertebrates such as fish and other wildlife) and sample matrices (e.g., carcass, liver, kidney, egg, feathers, etc.) for exposure to environmental contaminants. EPA will be guided by the following principles when using biological sampling data for sorting and priority setting.

a. Greater weight is generally given to data sets that provide relevant information on large populations, disproportionately exposed subpopulations, or particularly susceptible subpopulations.

b. Greater weight is generally given to non-detect data when it is associated with low analytical detection limits for organisms that are likely to be exposed.

ii. *Environmental, occupational, consumer product, and food-related data*. Environmental, occupational, consumer product, and food-related data include: Monitoring data for chemical contaminants found in a variety of environmental media to which humans and animals are exposed, such as water (surface, ground, and drinking), air, soil, sediment, and food; and use information for chemicals, when it is available. EPA will be guided by the following principles when using environmental, occupational, consumer product, and food-related data for initial sorting and priority.

a. Greater weight is generally given to validly measured data than to estimates.

b. Greater weight is generally given to data that demonstrate that a chemical is more likely to be internalized by an organism from its environment.

c. Greater weight is generally given to data sets that provide relevant information on large populations, disproportionately exposed subpopulations, or particularly susceptible subpopulations.

d. Greater weight is generally given to non-detect data when it is associated with low analytical detection limits for organisms that are likely to be exposed.

In the absence of monitoring data, estimates from the National Occupational Environment Survey, Permissible Exposure Limits (PELs) and similar estimates will be used to infer potential exposure levels. These estimates are much less robust than monitoring data but will be used unless actual monitoring data are submitted.

iii. *Environmental releases*. Environmental release information includes data on chemicals released to the environment to which humans and environmental species may be exposed, such as permitted industrial discharges to air or water and accidental release or spill data. EPA may use data from its Toxic Release Inventory (TRI) and the Agency for Toxic Substances Disease Registry's (ATSDR's) Hazardous

Substance Emergency Surveillance System. EPA will be guided by the following principles when using environmental release data for sorting and priority setting.

a. Greater weight is generally given to validly measured data than to estimates.

b. Greater weight is generally given to data demonstrating that an environmental release will more likely lead to organism exposure. (e.g., EPA will give greater weight to TRI releases to air and water than TRI releases to permitted landfills, etc.).

c. Greater weight is generally given during priority setting to data sets that provide relevant information on large populations, disproportionately exposed subpopulations, or particularly susceptible subpopulations.

iv. *Production volume data.* Production volume data are generally available for existing chemicals, but not for polymers, inorganics, or chemicals under 10,000 pounds of annual production. (These latter substances have been exempted from EPA's quadrennial TSCA Inventory Update Rule (40 CFR part 710, subpart B)). For new chemicals, the only production volume information available is estimates and it is not relevant for environmental contaminants. EPA will be guided by the following principles when using production volume data for sorting and priority setting.

a. Production volume provides only a very rough indication of potential human and environmental exposure.

b. Production data generally should be combined with other data (e.g., use and physical properties data) in an effort to minimize some of the inherent weaknesses of using production data as a surrogate for exposure.

c. Production information generally should not be used to compare existing industrial chemicals, pesticides and new chemicals because production volume ranges are too divergent. For example, production volumes for high-volume industrial chemicals are several orders of magnitude higher than those for either new chemicals or pesticides.

v. *Fate and transport data and models.* The fate and transport information category includes chemical and/or physical properties that may be used to predict or estimate the medium or media where a chemical is likely to be found and whether or not a chemical is likely to remain in the environment over time.

Environmental fate and transport information is available from various reference sources, including data bases, textbooks, and monographs. Numerous sources of data and models are listed in Appendix G of the EDSTAC Final Report (EDSTAC, 1998). The sheer volume of environmental fate and transport data makes it necessary to identify those data useful for sorting and prioritization purposes. EPA will focus attention on three subcategories of environmental fate and transport information including: Persistence, mobility, and bioaccumulation.

EPA will consider the following characteristics of fate and transport data: Hydrolysis half-life persistence; biodegradation persistence; photooxidation persistence; volatility (Henry's Law) mobility; adsorption coefficient (K_{oc}) mobility; and octanol: water partition coefficient (K_{ow}/LogP) mobility and bioaccumulation. EPA may use a multimedia fate and partitioning model to combine this information in a meaningful manner. EPA will be guided by the following principles when using fate and transport data and models for initial sorting and priority.

a. Air, water, and soil environmental compartments generally should be considered when using fate and transport data to help set priorities for screening.

b. Greater weight generally should be given to fate and transport characteristics based on laboratory or field tests than on estimates.

2. *Effects-related information and criteria.* EPA generally plans to rely on HTPS data, toxicological laboratory studies, epidemiological studies, and predictive structure activity models to assist the Agency in setting priorities for screening.

i. *Toxicological and epidemiological studies.* Toxicological laboratory studies include information related to the laboratory study of toxic effects of commercial chemicals, pesticides, contaminants, or mixtures on living organisms or cell systems including humans, wildlife, or laboratory animals. Epidemiological and field studies range from hypothesis-generating descriptive studies, such as case reports and ecological field analyses, to prospective cohort studies and rigorously controlled hypothesis-testing clinical trials.

Empirical toxicological and epidemiological data are reported in numerous peer-reviewed scientific journals. Published studies are conducted and described in varying degrees of methodological rigor and data are reported in widely varying detail. To rely on this information, EPA would be required to review it and determine its applicability and adherence to generally acceptable investigatory practices. The search and review of this primary literature would be too resource intensive to be part of the prioritization process. Instead EPA will rely on data bases containing studies addressing the endpoints of interest. In response to EPA's proposed *Priority List*, public commenters can submit studies that EPA will review. If the submitted studies indicate that the priority should be changed or they meet the requirements of portions of Tier 1, EPA will change the priority or screening requirements for that chemical, as appropriate.

EPA will be guided by the following principles when evaluating toxicological and epidemiological data:

a. Negative epidemiological studies generally will not override positive toxicological studies. Positive epidemiological studies generally will override negative toxicological studies for priority-setting purposes.

b. EPA generally will give greater weight to *in vivo* studies with relevant endpoints than to *in vitro* studies.

ii. *Predictive structure-activity models.* Predictive biological activity or effects models attempt to identify the correlation between chemical structure and biological activity, including those that can be identified through *in vitro* and *in vivo* screens. Models can be useful when biological data are unavailable. While EPA believes this approach will be of limited success early in the screening program, it believes that the refinement of models as more screening results become available may increase their utility as a predictive tool for priority setting and may actually replace some of the more mechanistic Tier 1 assays.

3. *Statutory criteria.* The FFDCA, as amended, requires that EPA provide for the testing of all ''pesticide chemicals.'' Under the FFDCA, ''pesticide chemical'' includes ''any substance that is a pesticide within the meaning of FIFRA, including all active and inert ingredients'' (21 U.S.C. 321(q)(1)). It also includes impurities. The statute does not restrict testing to pesticides used on foods. As part of priority setting, EPA will ensure that all substances that must be tested pursuant to the FFDCA—i.e., pesticide chemicals—are tested in a timely manner.

D. Use of a HTPS to Assist Priority Setting

For the majority of chemicals, EPA does not believe that any endocrine-disruptor effects data exists. This lack of data makes it difficult to set priorities for screening and testing. To help solve this problem, EPA plans to conduct two of the Tier 1 screening tests (see Units V.A. and VI.B. and C. of this notice) on approximately 15,000 chemicals in a high-speed, automated fashion. Since these assays are being run before the

Tier 1 screening is conducted, EPA refers to this testing as HTPS. HTPS test results will provide information on the interaction of chemicals with the estrogen and androgen receptor. The automated, low-cost nature of HTPS allows EPA to test a large number of chemicals in a short period of time. HTPS will provide EPA with preliminary information relating to one of several possible mechanisms by which a chemical may affect the endocrine system. Thus, EPA will use HTPS to assist in setting priorities for further screening; the Agency will not use HTPS alone to decide whether a chemical should or should not move to the next phase in the EDSP.

E. Setting Priorities for Tier 1 Screening

EPA plans to use existing, available information, HTPS data, and the EDPSD to establish Tier 1 screening priorities. EPA anticipates, however, that the quantity and quality of exposure and effects information will be uneven for the majority of chemicals. Thus, to ensure the integrity of the priority-setting process and avoid an ''apples'' to ''oranges'' comparison, EPA plans to adopt a ''compartment-based approach'' to priority setting. The term ''compartment'' refers to the particular information category or criterion or combinations of information or criteria that defines a set of chemicals, just as a group of parameters defines a set of numbers in mathematics. All members of the set must possess the properties required for membership in the compartment and thus will have these elements in common as the basis for comparison. Operationally, EPA will establish a limited number of compartments and sort chemicals into those compartments based on the criteria defining each compartment. EPA will then prioritize chemicals within each of the compartments according to criteria related to those for membership in the compartment. Finally, EPA will recombine the highest priority chemicals in each compartment to form the group of chemicals going into phase 1 of the screening program.

EPA has not identified all of the specific compartments. Examples of compartments, however, may include HPVCs, chemicals in consumer products, chemicals found in biological tissue, pesticide-active ingredients, formulation ingredients in pesticides, and chemicals found in sources of drinking water. A chemical could fall into more than one compartment. To help develop the list of priority-setting compartments, EPA plans to convene a priority-setting workshop for multi-stakeholders. The document announcing the priority-setting workshop is published elsewhere in this issue of the **Federal Register**.

Pesticides present a special difficulty in priority setting because data on both inert formulation ingredients and active ingredients need to be available at the time of a pesticide's evaluation. This will present some logistical difficulties in prioritizing the screening of pesticide formulations since pesticides with the same active ingredient may contain significantly different formulation inert ingredients.

Although EPA has not identified all priority-setting compartments, it has decided on some compartments. EPA plans to have a ''mixtures'' compartment, a ''naturally occurring non-steroidal estrogen'' compartment; and a ''nominations'' compartment. Each of these compartments is described in detail in this unit.

1. *Nominations*. The priority-setting process generally will give high priority to chemicals with widespread exposure at the national level. However, there are chemicals that result in disproportionately high exposure to identifiable groups, communities, or ecosystems. For these, EPA plans to establish process by which affected citizens can nominate chemicals with regional or local exposure to receive priority for Tier 1 screening (see Unit VI.E. of this notice).

2. *Mixtures*. Mixtures, defined as a combination of two or more chemicals, will need special attention during the initial stages of sorting and prioritization because they present unique challenges for testing and hazard assessment. Consequently, EDSTAC recommended that EPA determine the technical feasibility and, where feasible, screen and test representative samples of mixtures from six distinct types of mixtures, including: Contaminants in human breast milk; phytoestrogens in soy-based infant formula; mixtures of chemicals commonly found at hazardous waste sites; pesticide/fertilizers mixtures; disinfection byproducts; and gasoline.

EPA will investigate the technical feasibility for screening and testing mixtures as recommended by EDSTAC. This will include an evaluation of whether it is possible to identify a reasonable number of representative samples of mixtures from each of the recommended six types of mixtures, as well as the ability to send the representative samples of mixtures through HTPS, Tier 1 screening, and Tier 2 testing depending on their physical properties, and validation and standardization of the results.

3. *Naturally occurring non-steroidal estrogens (NONEs)*. Another special class of chemicals of interest to EPA are naturally occurring NONEs. These are natural products derived from plants (phytoestrogens) and fungi (mycotoxins). These chemicals occur widely in foods and have the potential to act in an additive, synergistic, or antagonist fashion with other hormonally active chemicals. EPA will work with the Food and Drug Administration (FDA) and the National Toxicology Program to obtain testing of the seven specific NONEs that were identified by EDSTAC.

F. Bypassing Tier 1 Screening

Recognizing the need for flexibility in applying the screening and testing requirements, EPA plans to permit chemicals to bypass Tier 1 screening under certain circumstances. If sufficient, scientifically relevant information exists regarding a specific chemical, EPA may move that chemical directly into Tier 2 testing. In addition, EPA may allow a chemical to bypass Tier 1 screening if the chemical's producer or registrant chooses to conduct Tier 2 testing without performing Tier 1 screening. Each of these two scenarios has different implications for the information requirements associated with completing Tier 2 testing.

1. *Chemicals that have previously been subjected to 2-generation reproductive toxicity tests*. This scenario includes chemicals that have previously been subjected to mammalian and wildlife developmental toxicology and/or reproductive testing, but where the tests did not include endocrine sensitive endpoints included in the most recent Office of Prevention, Pesticides, and Toxic Substances (OPPTS) or Organization for Economic Cooperation and Development (OECD) test guidelines (See Tables 2, 3, and 4 in Unit V.B. of this notice). Food-use pesticides fall into this category, as do a small number of certain other pesticides and industrial chemicals. Chemicals and non-food-use pesticides that meet this criterion also will likely be candidates for alternative approaches to Tier 2 testing.

Chemicals that have data from tests that meet the requirements of the new mammalian guidelines, but not the new wildlife tests, would be subjected to the wildlife testing requirements unless scientifically sound reasons are provided to limit testing.

2. *Chemicals for which there is limited prior toxicology testing*. The second bypass scenario includes chemicals whose manufacturer or

registrant has decided to voluntarily complete Tier 2 testing without having completed the full Tier 1 screening battery or any prior 2-generation reproductive toxicity testing. Chemicals that bypass Tier 1 screening under this scenario must be evaluated using the entire Tier 2 battery (i.e., the mammalian and non-mammalian multi-generation tests with all the recommended test species and endpoints) unless scientifically sound reasons are provided to limit testing.

EPA will generally follow the guidance set forth in this unit when setting Tier 2 testing priorities for chemicals that bypass Tier 1 screening:

i. If a chemical is deemed to be high priority for Tier 1 screening and the manufacturer or registrant of the chemical decides to voluntarily bypass Tier 1, it should also be high priority for Tier 2 testing. Voluntary action on the part of registrants/manufacturers should expedite testing.

ii. To the extent practicable, pesticides should be tested on the schedule EPA has established for tolerance reassessments, pesticide re-registration and registration renewal under the FFDCA and FIFRA, unless HTPS or other data indicate that the pesticide should be tested in a shorter timeframe. EPA does not intend to delay tolerance reassessments, re-registration or registration renewal actions to await implementation the EDSP.

G. Mixtures

For purposes of the EDSP, EPA defines ''mixture'' as a combination of two or more chemicals. EPA will consider most commercial chemicals (class 1 and class 2 substances under TSCA) to be chemicals even though they may contain other substances in them as impurities or exist as complex reaction products. In some cases a commercial product is in reality a complex mixture of unidentified composition in which no single substance predominates. These complex products have Chemical Abstract Service (CAS) numbers and will be regarded as chemicals from a legal and policy perspective but may need to be treated as mixtures from a scientific perspective in the EDSP. This determination will be made case by case.

EPA recognizes that the science of evaluating mixtures remains complex and unclear, but believes that it should begin to confront the issues raised by them. EPA will sponsor some screening of mixtures after the demonstration of the HTPS and validation of the Tier 1 screening battery on single chemicals.

Initially, EPA plans to include a few mixtures in the HTPS. EDSTAC has recommended that one or more representative samples from each of the following high priority mixtures would be tested:

1. Contaminants in human breast milk.
2. Phytoestrogens in infant soy formula.
3. Mixtures of chemicals found at hazardous waste sites.
4. Pesticide and fertilizer mixtures.
5. Disinfection byproducts.
6. Gasoline.

EPA also plans to evaluate some mixtures in the Tier 1 screen. If results of Tier 1 are positive for a mixture, the Agency will face a choice of testing the mixture in Tier 2 or determining what substances, or combination of substances, are responsible for the activity. The Agency likely will choose this latter course of action and test the individual active chemical or active fraction in Tier 2.

H. Categories of Chemicals

In its first TSCA proposed test rule (45 FR 48524, July 18, 1980), EPA outlined three approaches for testing chemicals belonging to a chemical category:

1. Test members of a category as individual chemicals.
2. Select test substances to represent the structural and chemical variation of the category as a whole.
3. Subdivide the category into subgroups and choose a representative from each as a surrogate for the entire subgroup.

For the HTPS, EPA plans to screen all members of a category that are produced in quantities over 10,000 pounds. The Agency will make a case-by-case decision regarding whether all of these chemicals will be required to go through Tier 1. However, it is likely that the HPVCs would be screened in Tier 1 regardless of the strategy used. As Quantitative Structure Activity Relationship (QSAR) modeling becomes more reliable, the two sampling approaches (approaches 2 and 3 as described in this unit) may become more viable alternatives.

V. Screening Program

EPA recognizes that a huge number of chemicals could be evaluated under the EDSP. EPA is adopting EDSTAC's recommendation of a two-tiered system to make the evaluation process more efficient. In Tier 1, a screening battery of assays will identify those chemical substances and mixtures capable of interacting with EAT. Tier 1 covers only screening tests and these alone are not sufficient to determine whether a chemical substance may have an effect in humans that is similar to an effect produced by naturally occurring hormones. The purpose of Tier 2 tests is to determine whether a chemical substance or mixture may cause endocrine-mediated effects for EAT, determine the consequences to the organism of the activities observed in Tier 1, and establish the relationship between the doses of the endocrine-active substance administered in the test and the effects observed.

A. Tier 1 Screening

Chemical substances or mixtures can alter endocrine function by affecting the availability of a hormone to the target tissue, and/or affecting the cellular response to the hormone. Mechanisms regulating hormone availability to a responsive cell are complex and include hormone synthesis, serum binding, metabolism, cellular uptake (e.g., thyroid), and neuroendocrine control of the overall function of an endocrine axis. Mechanisms regulating cellular response to hormones are likewise complex and are tissue specific. Because the role of receptors is often crucial to cellular responsiveness, specific nuclear receptor binding assays are included. In addition, tissue responses that are particularly sensitive and specific to a hormone are included as endpoints for Tier 1 screens. In order for the Tier 1 screening battery to discriminate between substances likely to affect the endocrine system and those not likely to affect it, the screening battery should meet the following criteria:

1. Detect all known modes of action for the endocrine endpoints of concern. All chemicals known to affect the action of EAT should be detected.
2. Maximize sensitivity to minimize false negatives while permitting a level of as yet undetermined, but acceptable, false positives. The screening battery should not miss potential EAT active materials.
3. Include a sufficient range of taxonomic groups among the test organisms. There are known differences in endogenous ligands, receptors, and response elements among taxa that may affect endocrine activity of chemical substances or mixtures. The screening battery should include assays from representative vertebrate classes to reduce the likelihood that important pathways for metabolic activation or detoxification of parent chemical substances or mixtures are not overlooked.
4. Incorporate sufficient diversity among the endpoints and assays to reach conclusions based on ''weight-of-evidence'' considerations. Decisions based on the screening battery results

will require weighing the data from several assays.

EPA's Tier 1 screening battery meets these criteria. The proposed Tier 1 screening battery and alternative assays for possible inclusion are:

Proposed Tier 1 Screening Battery

In Vitro

1. Estrogen Receptor (ER) Binding/Transcriptional Activation Assay.
2. Androgen Receptor (AR) Binding/Transcriptional Activation Assay. [1]
3. Steroidogenesis Assay with Minced Testis.

In Vivo

1. Rodent 3-Day Uterotrophic Assay (Subcutaneous (sc)).
2. Rodent 20-Day Pubertal Female Assay with Thyroid.
3. Rodent 5–7–Day Hershberger Assay.
4. Frog Metamorphosis Assay.
5. Fish Gonadal Recrudescence Assay.

Alternative Assays for Possible Inclusion in Tier 1

In Vitro

1. Placental Aromatase Assay.

In Vivo

1. Modified Rodent 3-Day Uterotrophic Assay (Intraperitoneal).
2. Rodent 14-Day Intact Adult Male Assay With Thyroid.
3. Rodent 20-Day Thyroid/Pubertal Male Assay.

EPA plans to include the alternative assays in the standardization and validation program. Combinations of the alternative assays, if validated and found to be functionally equivalent, could potentially replace three of the component assays in the recommended Tier 1 screening battery (*in vitro* steroidogenesis assay with testis, 20-day pubertal female assay, and 5–7-day Hershberger assay), thereby possibly reducing the overall time, cost, and complexity while maintaining equivalent performance of the overall Tier 1 screening battery.

1. *In vitro assays*. EPA has identified two categories of *in vitro* assays that may be used in Tier 1 screening to assess the binding of test substances to receptors, i.e., cell-free assays for receptor binding and transfected cells designed to detect transcriptional activation. The specific assays chosen, whether done ''at the bench'' or as a HTPS should have the following characteristics:

a. Evaluate binding to estrogen and androgen nuclear receptors.

b. Evaluate binding to the receptor in the presence and absence of metabolic capability (e.g., one or more of the P450 isozymes, e.g., cyp1A1, cyp3A4).

c. Distinguish between agonists and antagonists in functional assays.

d. Yield dose responses for relative potency of chemical substances or mixtures exhibiting endocrine activity.

In vitro evaluations can provide both false positive and false negative results. *In vitro* false positives (i.e., active *in vitro* but not *in vivo*) arise when a chemical is not absorbed or distributed to the target tissue, is rapidly metabolically inactivated and/or excreted, and/or when some other form of toxicity predominates *in vivo*. False negatives are considered to be of greater concern if *in vitro* tests were used to the exclusion of *in vivo* methods. *In vitro* evaluations can result in false negatives due to their inability, or diminished capacity, to metabolically activate toxicants. As a result, EPA's proposed screening battery includes *in vivo* methods in conjunction with *in vitro* techniques. Nevertheless, some *in vitro* assays may offer distinct advantages over *in vivo* assays when investigating the activity of specific metabolites.

The estrogen and androgen receptor binding assays provide an indication of the potential of a substance to disrupt ER or AR function *in vivo*. In the receptor binding assays the test chemical competes for binding at the receptor with the natural ligand or other strongly binding substance. EPA strongly prefers stably transfected transcriptional-activation assays over receptor binding assays. In addition to binding, there is a consequence to the binding with the transcriptional-activation assay, i.e., transcription (synthesis of messenger Ribonucleic Acid (mRNA)) of a reporter gene and translation of the mRNA to an identifiable detectable protein such as firefly luciferase or beta-galactosidase. This assay can distinguish between agonists and antagonists and can be run with and without metabolic activation.

The third *in vitro* assay in the screening battery is the steroidogenesis assay. This assay utilizes minced testes and detects the ability of substances to interfere with the endocrine system by inhibiting the activity of P450 enzymes in the steroid pathway. Inhibition of mammalian-steroid synthesis can potentially result in a broad spectrum of adverse effects *in vivo*, including abnormal serum hormone levels, pregnancy loss, delayed parturition, demasculinization of male offspring, lack of normal male and female mating behavior, altered estrous or menstrual cyclicity, and altered reproductive organ sizes and weights. Interference with other enzymes involved in the synthesis of specific hormones will be detected in the *in vivo* assays.

2. *In vivo assays*. The value of each individual assay cannot be considered in isolation from the other assays in the screening battery, as they have been combined in a manner such that limitations of one assay are complemented by strengths of another. *In vivo* assays complement *in vitro* assays in several important ways. *In vivo* methods in Tier 1 can help reduce false negatives related to absorption, distribution, metabolism, and excretion of a chemical substance in the absence of knowledge of its pharmacokinetics. *In vivo* assays typically cover a broader range of mechanisms of action than *in vitro* assays. It would be impractical to try to include an *in vitro* assay for every mechanism of action and in some cases it would be impossible as the mechanism would be expressed only in whole animal systems. It is clear that a combination of *in vivo* and *in vitro* assays is necessary in order to detect EAT alterations that act via the ER, AR, thyroid receptor (TR), inhibition of steroid hormone synthesis, and/or alterations of the hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-thyroid (HPT) axes. The screening battery, once validated, should detect all chemicals with the potential to disrupt the EAT systems, including xeno(anti)estrogens (that act via the ER or inhibition of aromatase by oral or parenteral administration), xeno(anti)androgens (via AR or hormone synthesis), altered HPG axis, and antithyroid action (via synthesis, metabolism and transport, and the TR). However, results of even the most specific *in vivo* assays can be affected by endocrine mechanisms other than those directly related to ER, AR, and TR action. The lack of specificity of *in vivo* assays is a limitation if the goal is to only identify ER, AR, and TR alterations. In contrast, this lack of specificity could be considered an advantage if a broader, more apical screening strategy is desired.

i. *Uterotrophic assay*. An increase in uterine weight is generally considered to be one of the best indicators of estrogenicity when measured in the ovariectomized (ovx) or immature female rat or mouse after 1–3 days of treatment. EPA is planning to require as part of the program a 3-day uterotrophic assay using the ovx adult female rat (the duration can be extended if so desired) with 10 animals per group. EPA will require sc treatment because most of the

[1] The ER and AR transcription activitation assays are in the HTPS. Those chemicals which go through the HTPS program, if it is technically feasible and validated, would not be required to separately undergo the first two *in vitro* assays at the bench.

historical data are collected in this manner and there are relatively few data concerning the effects of other routes of administration at this time. EPA is also planning to use this assay to detect antiestrogens. When run to detect antiestrogens, a control and xenobiotic-treated group are co-administered with estradiol. The uterotrophic assay is an *in vivo* check on the ER binding and ER reporter gene assays.

ii. *20-Day pubertal female with thyroid*. The 20-day pubertal female assay is the most comprehensive assay in the screening battery. It can detect thyroid effects, aromatase inhibitors, estrogens, antiestrogens, and agents which interfere with one of the hormone feedback loops that controls maturation and reproduction, the HPG axis. Next to *in utero* development, the pubertal stage is the most sensitive and vulnerable life stage.

Exposure of weanling female rats to environmental estrogens can result in alterations of pubertal development (Ramirez and Sawyer 1964). Exposure to a weakly estrogenic pesticide after weaning and through puberty induces pseudoprecocious puberty (accelerated vaginal opening without an effect on the onset of estrous cyclicity) after only a few days of exposure (Gray et al. 1989). Pubertal alterations are also observed in girls exposed to estrogen-containing creams or drugs, which induce pseudoprecocious puberty and alterations of bone development (Hannon et al. 1987).

In the pubertal female assay, oral dosing is initiated in weanling rats at 21 days of age (10 per group, selected for uniform body weights at weaning to reduce variance). The animals are dosed daily, 7 days a week, and examined daily for vaginal opening (one could also check for age at first estrus and onset of estrous cyclicity). Dosing continues until vaginal opening is attained in all females (typically 2 weeks after weaning, unless delayed). The advantage over the uterotrophic assay is that one test detects both agonists and antagonists, it detects xenoestrogens like methoxychlor that are almost inactive via sc injection, it detects aromatase inhibitors, altered HPG function, and unusual chemicals like betasitosterol. In addition, at necropsy one should weigh the ovary (increased in size with aromatase inhibitors, but reduced with betasitosterol), save the thyroid for histopathology, take serum for T4, and measure thyroid-stimulating hormone (TSH). In addition to estrogens, the age at vaginal opening and uterine growth can be affected by alteration of several other endocrine mechanisms, including alterations of the HPG axis (Shaban and Terranova 1986; and Gonzalez et al. 1983). In rats, this event can also be induced by androgens (Salamon 1938; and EGF (Nelson et al. 1991). In the last 20 years there have been over 200 publications which demonstrate the broad utility of this assay to identify altered estrogen synthesis, ER action, growth hormone, prolactin, follicle-stimulating hormone (FSH) or luteinizing hormone (LH) secretion, or central nervous system (CNS) lesions.

iii. *Rodent 5–7 day Hershberger assay*. This assay is designed to detect androgenic and antiandrogenic effects. In this *in vivo* assay, sex accessory gland weights (ventral prostate and seminal vesicle separately) are measured in castrated, T-treated adult male rats after 4–7 days of treatment by gavage with the test compound. The advantage of this assay is that it is fairly simple, short term, and relatively specific for direct androgenic/antiandrogenic effects compared to other *in vivo* procedures. To detect both agonists and antagonists the assay requires two-dosing regimes:

a. Castrated male rat + Xenobiotic (to detect agonist)

b. Castrated male rat + T + Xenobiotic (to detect antagonist)

Although the androgens, T, and dihydrotestosterone (DHT), play a predominant role in the growth and maintenance of the size of these accessory gland structures, several other hormones and growth factors can influence sex organ weights including the thyroid and growth hormones, prolactin, and epidermal growth factor (EGF). Exposure to estrogenic pesticides can also reduce sex accessory gland size; however, it is unclear to what degree these reductions result from direct versus indirect action of the chemical. Other useful endpoints that help reveal the mechanism of action include serum hormone levels of T, DHT, LH, AR distribution, TRPM2/C3 gene activation, ornithine decarboxylase (ODC), and 5-alpha-reductase activity in the prostate.

The prostate and seminal vesicles should be weighed separately because these organs differ with respect to the androgen that controls their growth and differentiation. The prostate is dependent upon enzymatic reduction of T to DHT, whereas the seminal vesicle is less dependent upon this conversion. Hence, effects on 5-alpha-reductase can be distinguished from AR-mediated mechanisms by determining whether the prostate is preferentially affected. Growth of the levator ani muscle is T dependent, having little capacity to convert T to the more potent androgen DHT. Weight of this muscle is useful in identifying anabolic androgens and antiandrogens, and for this reason has been used extensively in the pharmaceutical industry. In order to detect androgenic rather than antiandrogen action one would simply delete the hormone administration from the protocol.

iv. *Frog metamorphosis assay*. This assay is in the screening battery to detect thyroid (increase in tail resorption rate) and antithyroid (decrease in tail resorption rate) effects. It also broadens the taxonomic representation of the screening battery. This assay employs intact larval (tadpole) stages of the African clawed frog (*Xenopus laevis*) exposed over a 14-day time period, 50–64 days of age, to observe the rate of tail resorption (Fort and Stover 1997). Tail resorption can be easily quantified with computer-aided video image processing (Fort and Stover 1997). The molecular mechanisms involved in tail resorption are well characterized (Brown et al. 1995; Hayes 1997a) and this assay is, therefore, considered to be a simple and specific assay for thyroid action. Because evidence also suggests that thyroid action on tail resorption is regulated by corticoids, estrogens, and prolactin (Hayes 1997b), this assay will address distinctive modulating pathways and, in tandem with the 20-day mammalian pubertal assay, a comprehensive screen for thyroid hormone activity is achieved.

v. *Fish gonadal recrudescence assay*. This assay is in the Tier 1 screening battery because as a group, fish are the most distant from mammals within the vertebrates, and it provides an additional safeguard that endocrine disruptors will not pass through the screen undetected. Intact mature fish maintained under simulated ''winter'' conditions (short-day length, cool temperatures) exhibit regressed secondary sex characteristics and gonad maturation.

In this assay, intact fish of both sexes (fathead minnow, *Pimephales promelas*, or other appropriate species) are simultaneously subjected to an increasing photoperiod/temperature regime and test substance to determine potential effects on maturation from the regressed position (recrudescence). The primary endpoints examined in the assay include morphological development of secondary sexual characteristics, ovary and testis development (weight increases), gonadosomatic index (ratio of gonadal weight to body weight), final gamete maturation (ovulation, spermiation), and induction of vitellogenin. This assay is sensitive to HPG axis effects in

addition to androgen- and estrogen-related activity.

Having diverse taxa in Tier 1 may give some information on the homology of the endocrine system across species and likelihood of consistent response across taxa and among organisms of the same species and when one must be concerned about variability.

3. *Alternative assays for possible inclusion*. These assays are being developed and validated (see Unit VI.F. of this notice) and may be acceptable cost effective substitutes for some of the assays in the primary Tier 1 screening battery of recommended by EDSTAC.

i. *Placental aromatase assay*. Aromatase converts T to estradiol. If an assay using a male is substituted for the 20-day pubertal female assay it will be necessary to add this assay to the screening battery since aromatase is present at very low levels in the testis. It is present at higher levels in the ovary, uterus, and placenta. Human placental aromatase is commercially available and could be used *in vitro* to assess the effects of toxicants on this enzyme.

ii. *Modified rodent 3-day uterotrophic assay (Intraperitoneal)*. The intraperitoneal (ip) injection method may enhance the sensitivity of the uterotrophic assay and is capable of detecting the estrogenic potential of methoxychlor, which has been cited as an example of a compound not detectable by the sc route. This is an *in vivo* assay (O'Conner et al. 1996) for estrogenic activity in ovx female rats. It can detect certain antiestrogens with mixed activity, i.e., some agonistic activity (e.g., tamoxifen).

The rats are injected intraperitoneally with the test agent daily for 3 days. The females are necropsied either 6 hours or 24 hours after the final treatment, depending on the protocol employed by the laboratory. Vaginal cytology is evaluated by vaginal lavage to determine whether the epithelium has become cornified, indicative of estrus. Presence of fluid in the uterine lumen is noted and recorded, and the number of animals that have fluid in the uterus is reported. Fluid imbibition (uptake) is indicative of estrogenic potential. The uterus is excised and weighed. It is then preserved in an appropriate fixative for subsequent histological evaluation, if needed. Subsequent histological evaluation will be triggered by an equivocal uterine weight or uterine fluid response (i.e., an increase that is not statistically significant). This evaluation will consist of a characterization of the appearance of the uterine epithelium, a measurement of uterine epithelial cell height, and epithelial mitotic index or proliferating cell nuclear antigen (PCNA) immunohistochemistry. Uterine cell height and cell proliferation are sensitive indicators of estrogenic potential.

iii. *14-Day intact adult male assay*. This *in vivo* assay is intended to detect effects on male reproductive organs that are sensitive to antiandrogens and agents that inhibit T synthesis or inhibit 5-alpha-reductase (Cook et al. 1997). The proponents of this assay believe that the duration of the assay is sufficient to detect effects on thyroid gland activity. The rats are anatomically intact and mature; therefore, they have an intact HPG axis, allowing an assessment of the higher order neuroendocrine control of male reproductive function and the thyroid. This assay coupled with the aromatase assay could potentially replace the Hershberger and the pubertal female assays in the recommended screening battery. Empirical assessment of this assay has shown it to be sensitive to agents that are directly antiandrogenic, inhibit 5-alpha-reductase, inhibit T synthesis, or affect thyroid function. The sensitivity of this assay, as defined as the ability to detect a hazard, may be comparable to other assays that have been recommended.

Young adult male rats (70–90 days of age) are used in this assay. They are dosed daily with the test agent for 14 days. The recommended route of administration is ip, which may, in some cases, maximize the sensitivity of the assay. They are necropsied 24 hours after the final dose. Immediately after sacrifice, one cauda epididymis is weighed and processed for evaluation of sperm motility and concentration. The following organs are weighed: Testes, epididymides, seminal vesicles, and prostate. The following are fixed and evaluated histologically: One testis and epididymis and the thyroid. The following hormones are measured in blood plasma: T4, TSH, LH, T, DHT, and estradiol.

iv. *Rodent 20-day thyroid/pubertal male assay*. This assay (in conjunction with the aromatase assay) is another candidate to replace the pubertal female and Hershberger assays in the screening battery. The thyroid/pubertal male assay detects androgens and antiandrogens *in vivo* in a single stage-apical test. ''Puberty'' is measured in male rats by determining age at preputial separation (PPS). Preputial separation and sex accessory gland weights are sensitive endpoints. However, a delay in PPS is not pathognomonic for antiandrogens. Pubertal alterations result from chemicals that disrupt hypothalamic-pituitary function (Huhtaniemi et al. 1986), and, for this reason, additional *in vivo* and *in vitro* tests are needed to identify the mechanism of action responsible for the pubertal alterations. For example, alterations of prolactin, growth hormone, gonadotrophin (LH and FSH) secretion, or hypothalamic lesions alter the rate of pubertal maturation in weanling rats. Sex accessory gland weights in intact-adult male rats also can be affected directly or indirectly by toxicant exposure. The HPG axis in an intact animal is able to compensate for the action of antiandrogens by increasing hormone production, which counteracts the effect of the antiandrogen on the tract (Raynoud et al. 1984; Edgren 1994; Hershberger 1953).

Delays in male puberty result from exposure to both estrogenic and antiandrogenic chemicals including methoxychlor (Gray et al. 1989), vinclozolin (Anderson et al. 1995b and dichlorodiphenyldichloroethylene (p,p' DDE) (Kelce et al. 1995). Exposing weanling male rats to the antiandrogenic pesticides p,p' DDE or vinclozolin delays pubertal development in weanling male rats as indicated by delayed PPS and increased body weight (because they are older and larger) at puberty. In contrast to the delays associated with exposure to estrogenic substances, antiandrogens do not inhibit food consumption or retard growth (Anderson et al. 1995). Antiandrogens cause a delay in PPS and affect a number of endocrine and morphological parameters including reduced seminal vesicle, ventral prostate, and epididymal weights. It is apparent that PPS is more sensitive than are organ weights in this assays. In addition, responses of the HPG are variable. In studies of vinclozolin, increases in serum LH were a sensitive response to this antiandrogen, whereas serum LH is not increased in males exposed to p,p' DDE during puberty (Kelce et al. 1997). Furthermore, a systematic review of the literature indicates that the sex accessory glands of the immature intact-male rat are consistently more affected than in the adult intact-male rat.

Animals are dosed by gavage beginning 1 week before puberty (which occurs at about 40 days of age) and PPS is measured. Androgens will accelerate and antiandrogens and estrogens will delay PPS. The assay takes about 3 weeks and allows for comprehensive assessment of the entire endocrine system in one study. The animals (10 per group, selected for uniform body weights to reduce variance) are dosed daily, 7 days a week, and examined daily for PPS. Dosing continues until 53

days of age; the males are then necropsied. The body, heart (thyroid), adrenal, testis, seminal vesicle plus coagulating glands (with fluid), ventral prostate, and levator ani plus bulbocavernosus muscles (as a unit) are weighed. The thyroid is retained for histopathology and serum is taken for T4, T3, and TSH. Testosterone, LH, prolactin, and DHT analyses are optional. These endpoints take several weeks to evaluate and are affected not only by estrogens but by environmental antiandrogens, drugs that affect the hypothalamic-pituitary axis (Hostetter and Piacsek 1977; Ramaley and Phares 1983), and by prenatal exposure to 2,3,78-tetrachlorodibenzo-p-dioxin (TCDD) (Gray et al. 1995a; Bjerke and Peterson 1994) or dioxin-like PCBs (Gray et al. 1995b). In contrast to these other mechanisms, only peripubertal estrogen administration accelerates this process in the female and delays it in the male. Preputial separation in the male rodent is easy to measure and this is not a terminal measure (Korenbrot et al. 1977). Age and weight at puberty, reproductive organ weights, and serum hormone levels can also be measured.

As indicated in this unit, the determination of the age at ''puberty'' in the male rat uses endpoints that already have gained acceptance in the toxicology community. Preputial separation in the male is a required endpoint in the new EPA 2-generation reproductive toxicity test guideline. In this regard, this assay would be easy to implement because these endpoints have been standardized and validated and PPS data are currently being collected under Good Laboratory Practice (GLP) conditions in most toxicology laboratories. In addition, PPS data are reported in many recently published developmental reproduction studies (i.e., see studies from R.E. Peterson's, J. Ashby's, R. Chapin's, and L.E. Gray's laboratories on dioxins, PCBs, antiandrogens, and xenoestrogens).

4. *Selection of doses in screening assays.* All *in vitro* screening assays (including the steroidogenesis assay) will involve multiple-dose levels, whether performed by HTPS or bench level methods, so a dose-response curve and assessment of relative potencies can be developed. EDSTAC recommended that *in vivo* screening assays be conducted at a single-dose level to save testing resources. In comments on the draft EDSTAC Report the SAB/SAP raised concern that relying on a single-dose level might give false negative results. EPA believes this question can be resolved in the standardization and validation program. EPA will require one-, two-, or three-dose levels for *in vivo* screens depending upon the results of the standardization and validation program. Information to assist in selecting the doses in the *in vivo* screens includes:

i. Prior information, such as that available during the priority-setting phase.

ii. Results from the HTPS (or its equivalent bench-level assays).

iii. Results from range-finding studies, utilized for T1S dose selection.

Results from the HTPS (or its equivalent) will provide potency information (i.e., EC 50) relative to a positive control such as 17-beta estradiol (E2), diethylstilbestrol (DES), or T for those chemical substances or mixtures which bind to the estrogen or androgen receptors. Information on the *in vitro* effective doses of E2, DES, or T, can be used to set the dose level(s), based on the validation process, for the *in vivo* Tier 1 screening assays for these chemical substances or mixtures.

It may be more cost effective to conduct the shortest of the *in vivo* screening assays at several doses without the intermediate step of a range finding study since repeating the study at different doses in the event that inappropriate doses are used would be relatively inexpensive. A range-finding study can be performed at multiple dose levels (at least five) with a few animals per dose level and a limited number of relevant endpoints. In general, range-finding studies should meet the following guidelines:

i. Use of the same species strain, sex(es), and age in the assay for which it is being performed (principal study).

ii. Use of the same route of administration, vehicle, and duration of dosing as in the principal study.

iii. Use of multiple dose levels; the number of dose levels will depend on the availability and extent of prior information.

iv. Use of multiple animals per dose level which may be fewer than the number used per group in the assay.

v. Use of relevant endpoints, which may be more limited than those in the main assay; for example, the range-finding study for the uterotrophic assay may employ only body weights and uterine wet weight, while the full screening assay may also evaluate uterine gland height, serum hormone levels, and/or vaginal cornification, etc.

vi. Use of comparable animals, e.g., ovarectomized females for the uterotrophic range-finding study or castrated males for the Hershberger range-finding assay. However, there may be circumstances under which exceptions occur, e.g., use of intact males in the range-finding study for the Hershberger assay to define doses producing systemic toxicity and any effects on the reproductive system as a first pass approximation.

vii. Use of more than one range-finding study if the initial version does not identify the dose level(s) to be used in the specific Tier 1 screening assay if necessary by extrapolation or interpolation.

The doses to be selected for the *in vivo* assays should not result in excessive systemic toxicity, but should result in effects useful for detection of potential EAT disruption. However, no-dose level higher than one gram/kilogram body weight/day (i.e., a ''limit'' dose) should be utilized. The rationale for selection of dose levels for each range-finding study, all of the results for such studies, and the logic employed to select the dose level(s) for the principal study should be included in the submission of study results for evaluation by the Agency as to the appropriateness of the study design, conduct, and conclusions.

B. Tier 2 Testing

The purpose of Tier 2 testing is to characterize the likelihood, nature, and dose-response relationship of the endocrine disruption of EAT in humans, fish, and wildlife. To fulfill this purpose, the tests are longer-term studies designed to encompass critical life stages and processes, a broad range of doses, and administration of the chemical substance by a relevant route of exposure, to identify a more comprehensive profile of biological consequences of chemical exposure and relate such results to the dose or exposure which caused them. Dose selection, specifically the use of environmentally relevent low doses for endocrine disruptor testing, has not been conclusively resolved. The EPA will continue its collaborations with other Federal agencies, industry, and environmental and public health organizations regarding low-dose research projects to resolve outstanding scientific questions. Effects associated with endocrine disruption may be latent and not manifested until later in life or may not appear until the reproductive period is reached. Unless a rationale exists to limit the test to 1 generation, tests for endocrine disruption will usually encompass 2 generations including effects on fertility and mating, embryonic development, sensitive neonatal growth and development, and transformation from the juvenile life stage to sexual maturity.

The outcome of Tier 2 is designed to be conclusive in relation to the outcome

of Tier 1 and any other prior information. Thus, a negative outcome in Tier 2 will supersede a positive outcome in Tier 1. Furthermore, each full test in Tier 2 has been designed to include those endpoints that will allow a definitive conclusion as to whether or not the tested chemical substance or mixture is or is not an endocrine disruptor for EAT in that species/taxa. Conducting all five tests in the Tier 2 testing battery would provide a more comprehensive profile of the effects a chemical substance or mixture could induce via EAT disruption mode(s)/mechanism(s) of action than would be the case if only a subset of tests or less comprehensive tests were performed. Considerations for determining whether the full battery of comprehensive tests should be implemented include an understanding of mechanisms of action, environmental fate and transport, persistence, potential for bioaccumulation, and potential exposure. EPA plans to require that all tests be performed in Tier 2 with all endpoints, unless compelling information is presented to show why testing should be limited.

Despite the design of Tier 2 to be as definitive as possible, there will always be situations in which ambiguous results are obtained. In some of these cases a weight of evidence approach using Tier 1 and Tier 2 data together may resolve the ambiguity. In others, it may be necessary to conduct additional special studies or to repeat a test to resolve the data interpretation issues.

1. *Tier 2 tests.* EPA is proposing that the Tier 2 test battery include the following tests: 2-Generation Mammalian Reproductive Toxicity Study, Avian Reproduction, Fish Reproduction, Amphibian Reproduction and Developmental Toxicity, and Invertebrate Reproduction.

Except for the amphibian reproduction and developmental toxicity study, these tests are routinely performed for pesticides with widespread outdoor exposures that are expected to affect reproduction. Modifications to each may be necessary to enhance the ability to detect endocrine-related effects. The amphibian test, though not standardized, is important because of the extensive fundamental knowledge base on amphibian development and the realization that amphibians may serve as key indicators of the health of the environment.

There is utility in considering the results of the entire battery when assessing human risk. For instance, if the results from different taxa produce similar results, one can feel more confident that the results are generally applicable to humans. If the results are widely divergent, either qualitatively or quantitatively, it indicates greater biological variability and perhaps additional caution in conducting a hazard assessment.

i. *Mammalian reproductive toxicity.* The 2-generation reproductive toxicity study in rats (40 CFR 799.9380; OPPTS Guideline 870.3800; OECD Guideline No. 416, 1983; FIFRA, Subdivision F, Guidelines 83–4) is designed to evaluate comprehensively the effects of a chemical on gonadal function, estrous cycles, mating behavior, fertilization, implantation, pregnancy, parturition, lactation, weaning, and the offspring's ability to achieve adulthood and successfully reproduce, through 2 generations, one litter per generation. While administration is usually oral (dosed feed, dosed water, or gavage), other routes are acceptable if justified (e.g., inhalation). In addition, the study also provides information about neonatal survival, growth, development, and preliminary data on possible teratogenesis.

In the existing 2-generation reproductive toxicity test, a minimum of three-treatment levels and a concurrent control group are required. At least 20 males and sufficient females to produce 20 pregnant females must be used in each group as prescribed in this current guideline. The highest dose must induce toxicity (or meet the limit dose requirement) but not exceed 10% mortality. In this study, potential hormonal effects can be detected through behavioral changes, ability to become pregnant, duration of gestation, signs of difficult or prolonged parturition, apparent sex ratio (as ascertained by anogenital distances) of the offspring, feminization or masculinization of offspring, number of pups, stillbirths, gross pathology and histopathology of the vagina, uterus, ovaries, testis, epididymis, seminal vesicles, prostate, and any other identified target organs.

Table 2 provides a summary of the endpoints evaluated within the framework of the experimental design of the updated 2-generation reproductive toxicity test (and some recommended additional endpoints for validation and inclusion to cover EAT concerns). These endpoints are comprehensive and cover every phase of reproduction and development. Tests that measure only a single dimension or component of hormonal activity, (e.g., *in vitro* or short-term assays) provide supplementary and/or mechanistic information cannot provide the breadth of information that is critical for risk assessment.

Additionally, in this study type, hormonally induced effects such as abortion, resorption, or premature delivery as well as abnormalities and anomalies such as masculinization of the female offspring or feminization of male offspring, can be detected. Substances such as the phytoestrogen, coumesterol, and the antiandrogen cyproterone acetate, which possess the potential to alter normal sexual differentiation, were similarly detected in this study test system (i.e., 1982 Guideline).

Table 2 contains two types of lists: First, those endpoints required in current EPA harmonized 1998 test guidelines; second, additional endpoints recommended by EDSTAC for validation and inclusion in both the recommended 2-generation test, as well as the alternative mammalian tests discussed in Unit V.B.3. of this notice. These additional endpoints will detect EAT effects.

The default assumption is that all of these endpoints would be evaluated unless the conditions which are set forth in the guidelines for determining the selection of endpoints are met.

Table 2.—Mammalian Tier 2 Test Endpoints

Current Guideline Endpoints Sensitive to Estrogens/Antiestrogens

sexual differentiation
gonad development (size, morphology, weight) ≤ accessory sex organ (ASO) development
ASO weight ± fluid; histology
sexual development and maturation: Acquisition of vaginal patency (VP), PPS
fertility
fecundity
time to mating
mating and sexual behavior
ovulation
estrous cyclicity
gestation length
abortion
premature delivery
dystocia
spermatogenesis
epididymal sperm numbers and morphology; testicular spermatid head counts; daily sperm production (DSP); efficiency of DSP
gross and histopathology of reproductive tissues
anomalies of the genital tract
viability of the conceptus *in utero* (prenatal demise)
survival and growth of offspring
maternal lactational behaviors (e.g., nursing, pup retrieval, etc.)

Current Guideline Endpoints Sensitive to Androgens/Antiandrogens

altered apparent sex ratio (based on AGD)
malformations of the urogenital system
altered sexual behavior
changes in testis and ASO weights
effects on sperm numbers, morphology, etc.
retained nipples in male offspring

altered AGD (now triggered from PPS/VP)
reproductive development; PPS/VP (puberty)
male fertility
agenesis of prostate
changes in androgen-dependent tissues in pups and adults (not limited to sex accessory glands)

Recommended Additional Estrogen/ Androgen Endpoints for Validation and Inclusion

ASO function (secretory products)
sexual development and maturation (nipple development and retention)
androgen and estrogen levels
LH and FSH levels
testis descent

Current Guideline Endpoints Sensitive to Thyroid Hormone

Agonists/Antagonists (general)
growth, body weight
food consumption, food efficiency
developmental abnormalities
perinatal mortality
testis size and DSP
VP; PPS

Recommended Additional Thyroid Endpoints for Validation and Inclusion

neurobehavioral deficits (see developmental landmarks in this unit)
TSH, T4, thyroid weight and histology (e.g., goiter)
developmental landmarks:
prewean includes pinna detachment, surface righting reflex, eye opening, acquisition of auditory startle, negative geotaxis, mid-air righting reflex, motor activity on PND 13, 21, etc.
postwean includes motor activity PND 21 and postpuberty ages (sex difference); learning and memory PND 60—active avoidance/water maze
brain weight (absolute), whole and cerebellum
brain histology

ii. *Avian reproduction test.* While birds are not included as subjects in the Tier 1 screening battery, it is important to evaluate the effects of exposure of birds to chemical substances or mixtures with endocrine activity.

EPA is planning to modify its Avian Reproduction Test guideline (OPPTS Guidelines 850.2300) for use in the endocrine disruptor testing program. The modification include: The additional endpoints presented in this unit to make the test more sensitive to chemical substances or mixtures with endocrine activity. Table 3 provides a summary of the endpoints evaluated within the framework of the Avian Reproduction Test (and recommended additional endpoints for validation and inclusion to cover EAT concerns). Two important extensions of this guideline include modification and standardization of the husbandry and dosing of the offspring from EPA's Avian Reproduction Test guidelines (OPPTS Guidelines 850.2300) to create a 2-generation avian reproduction test and evaluation of an additional exposure pathway (i.e., direct topical exposure, which is common in the wild, by dipping eggs). The extensions to the guideline are outlined in Appendix Q in the EDSTAC Final Report (EDSTAC, 1998).

In the current Avian Reproduction Test guidelines, two species are commonly used, mallards and northern bobwhite. Exposure of adults begins prior to the onset of maturation and egg laying and continues through the egg-laying period; their offspring are exposed, in early development, by material deposited into the egg yolk by the females. These offspring can be used efficiently to test for the effects of chemical substances or mixtures on avian development. There are several endpoints currently required (see OPPTS Guidelines 850.2300(c)(2)) that are particularly relevant to disruption of endocrine activity, including: Eggs laid, cracked eggs, eggshell thickness, viable embryos, and chicks surviving to 14 days. EPA is extending the guidelines to require: Additional measurements of circulating steroid titers, thyroid hormones, major organ (including brain) weights, gland weights, bone development, leg and wing bone lengths, and ratios of organ weights to bone measurements; skeletal x-rays; histopathology; functional tests; and assessment of reproductive capability of offspring (Baxter et al. 1969; Bellabarba et al. 1988; Dahlgren and Linder 1971; Emlen 1963; Cruickhank and Sim 1986; Fleming et al. 1985a; Fleming et al. 1985b; Fox 1976; Fox et al. 1978; Freeman and Vince 1974; Hoffman and Eastin 1981; Hoffman and Albers 1984; Hoffman 1990; Hoffman et al. 1993; Hoffman et al. 1996; Jefferies and Parslow 1976; Kubiak et al. 1989; Maguire and Williams 1987; Martin 1990; Martin and Solomon 1991; McArthur et al. 1983; McNabb 1988; Moccia et al. 1986; Rattner et al. 1982; Rattner et al. 1987; Summer et al. 1996; Tori and Mayer 1981).

Table 3.—Avian Reproduction Test Endpoints

Current Guideline Endpoints Sensitive to Estrogens/Antiestrogens, Androgens/ Antiandrogens, and/or HPG Axis

egg production
eggs cracked
viable embryos (fertility)
eggshell thickness
fertilization success
live 18-day embryos
hatchability
14-day-old survivors

Recommended Additional Endpoints for Validation and Inclusion

sex ratio
major organ (including brain) weights
gland weights
histopathology
plasma steroid concentrations
neurobehavioral test (e.g., nest attentiveness)

Current Guideline Endpoints Sensitive to Thyroid Hormone Agonists/Antagonists

body weight of adults
food consumption of adults
body weight of 14-day-old survivors
developmental abnormalities

Recommended Additional Endpoints for Validation and Inclusion

plasma T3/T4
thyroid histology
bone development (skeletal x-ray)
ratio of organ weights to bone measurements
neurobehavioral test (cliff test)
cold stress test

iii. *Fish reproduction test.* Fish are the most diverse of all vertebrates. Reproductive strategies extend from oviparity, to ovoviviparity, to true viviparity. The consequences of an endocrine disruptor may be quite different across the many families of fishes. As a first step though, EPA plans to require use of fathead minnows, or in special cases, sheepshead minnows in the Fish Life Cycle Test. The Fish Life Cycle Test consists of continuous exposure from fertilization through development, maturation, and reproduction, and early development of offspring with a test duration of up to 300 days. EPA also anticipates use of the fathead minnow in the Tier 1 fish gonadal recrudescence assay, and as such, the relevance of any activity detected in the screening assay would be evaluated. If exposure to a particular chemical substance or mixture is predominantly estuarine or marine, EPA may require use of the estuarine sheepshead minnow (*Cyprinodon variegatus*) in the test. However, EPA will permit flexibility to species selection with appropriate justification as to species choice by the test sponsor.

The Fish Life Cycle Test (OPPTS 850.1500) follows procedures outlined in (Benoit 1981) for the fathead minnow and (Hansen et al. 1978) for the sheepshead minnow. In general, the test begins with 200 embryos distributed among eight incubation cups in each treatment group. When hatching is completed, the number of larvae are reduced to 25 individuals, if available, which are released to each of four replicate larval growth chambers. Four weeks following their release into the larval growth chambers, the number of juvenile fish are reduced again and 25 individuals, if available, distributed to each of two replicate adult test chambers. When fish reach sexual

maturity, fish are separated into spawning groups (pairs or one male/two females) with a minimum of eight breeding females. Remaining adults will be maintained in the tank but will be segregated from the spawning groups. Adults will be allowed to reproduce, at will, until the 300th day of exposure. Alternatively, the test may be continued past 300 days until 1 week passes in which no eggs from any group have been laid. The embryos and fish are exposed to a geometric series of at least five test concentrations, a negative (dilution water) control, and, if necessary, a solvent control.

Assessment of effects on offspring of the parental group (first filial or F1 generation) will be made by collecting two groups of 50 embryos from each experimental group and incubating those embryos. When embryos hatch, the number of larvae hatched from each group will be impartially reduced to 25, if available, and released into the larval growth chambers. After 4 weeks of exposure, lengths, and weights of surviving individuals will be recorded.

Observations are made of the effects of the test substance on embryo hatching success, larvae-juvenile-adult survival, growth of parental and F1 generation, and reproduction of the adults. Table 4 provides a summary of the endpoints evaluated within the framework of the Fish Life Cycle Test (and recommended additional endpoints for validation and inclusion to cover EAT concerns).

Table 4.—Fish Reproduction Test Endpoints

Current Guideline Endpoints Sensitive to Estrogens/Antiestrogens, Androgens/ Antiandrogens, and/or HPG Axis

viability of embryos
time to hatch
spawning frequency
egg production
fertilization success

Recommended Additional Endpoints for Validation and Inclusion

sexual differentiation (tubercle formation, gonadal histology)
sex ratio
gonadosomatic index
gamete maturation (production, final oocyte maturation, sperm motility test, etc.)
vitellogenin
plasma steroid concentrations
in vitro gonadal steroidogenesis

Current Guideline Endpoints Sensitive to Thyroid Hormone Agonists/Antagonists

growth, length, and body weight
developmental abnormalities

Recommended Additional Endpoints for Validation and Inclusion

plasma T3/T4
thyroid histopathology
bone development (skeletal x-ray)
ration of organ weights to bone measurements
neurobehavioral test (cliff test)
cold stress test

iv. *Invertebrate reproduction test.* Although invertebrates do not generate EAT, EPA plans, through use of this test, to examine in more depth invertebrate hormones that are functionally equivalent to EAT. The species of choice would be mysids or daphnia.

Although neither the daphnia nor the mysid chronic test was designed to examine endocrine-specific endpoints, both species are crustaceans and therefore share common physiology. Ecdysone is a steroid hormone that regulates growth and molting in arthropods, and exhibits some functional and structural similarities to estrogen. The central role of ecdysone makes it an attractive candidate for examining endocrine effects in invertebrates; however, other possibilities also exist. Morphogenetic and reproductive development of arthropods is controlled in part by juvenile hormone (JH). Methyl farnesoate is a JH like compound that may play a role in reproduction and development (Borstet et al. 1987; Laufer et al. 1987a,b).

Invertebrate hormones are beyond the immediate scope of the EDSTAC which has focused on the vertebrate EAT. Nevertheless, invertebrate hormones that are functionally equivalent to EAT need to be examined in more depth. More importantly, chemicals that affect these vertebrate hormones may also affect invertebrate hormones resulting in altered reproduction, development, and growth.

Chemicals with estrogenic properties are reported to have altered normal function of ecdysone systems (Mortimer 1993, 1994, 1995a, 1995b; Chu et al. 1997). Satyanarayana et al. 1994 showed stimulation of vitellogenin in insect prepupae and pupae by methoprene, a JH mimic with retinoid properties. Whether vitellogenin production is controlled through either an estrogen receptor or an alternative mechanism is not crucial for obtaining test results that show alteration occurs.

Therefore, the mysid shrimp chronic life cycle test (OPPTS 850.1350) may be adapted to determine whether chemicals that affect hormonal activity in vertebrates also affect arthropods. Once adapted to include reproductive and developmental endpoints relevant to the EDSP, the test could be a useful component in screening and testing.

The other common invertebrate bioassay, one using the water flea, daphnia, is used internationally (OECD Guideline No. 202). It incorporates life cycle assessment and reproductive and developmental endpoints, albeit applied quite differently in this group of animals. Reproduction is usually parthenogenic in the laboratory in these animals, limiting the applicability to endpoints identified in this report. The particular aspect of this system is that the daphnia is sensitive to estrogenic compounds (Baldwin et al. 1995; Baldwin et al. 1997; Shurin and Dodson 1997), and possesses receptors for T, making the system sensitive to another vertebrate hormone. Again, this bioassay would have to be adapted for the endpoints and processes of interest in the EDSP as a protocol for including invertebrate species in the endpoints addressed by the EDSP screening and testing batteries. Other invertebrates, such as molluscs, crayfishes, and echinoderms, do have EAT, but again relevant standardized tests for evaluating the consequences of interfering with these systems are not currently available. It is simply not known whether one (mysid) or two (mysid and daphnia) Tier 2 tests will provide sufficiently valid information for other invertebrate groups not tested. This is a source of uncertainty, potentially leading to Type II errors of unknown magnitude. These issues will be addressed during the development and validation of this assay.

v. *Amphibian development and reproduction.* A definitive amphibian test, which exposes larvae through metamorphosis and reproduction, is important to evaluate the consequences of endocrine disruption in poikilothermic oviparous vertebrate distinct from fishes. A rich literature on metamorphosis, growth, and reproduction exists for frogs. No established method has been identified which is suitably comprehensive to serve as a Tier 2 test at this time but a promising method is under development by EPA.

2. *Alternative test procedures*—i. *Alternative Mammalian Reproduction Test (AMRT).* One alternative to the 2-generation test procedure in Unit V.B.1.i. of this notice is the AMRT. The objectives of this test are to describe the consequences of *in utero* and/or lactational exposure on reproduction and development from compounds that displayed EAT activity in the Tier 1 screens. If validated, this test may be used, under certain defined circumstances, instead of the recommended 2-generation reproductive toxicity test (TSCA guidelines, 1997) in Tier 2 tests. In this regard, the test will be conducted with

at least three treatment groups plus a control and include endpoints sensitive to chemicals that alter development via EAT activities. As with the 2-generation mammalian reproductive toxicity study, the default assumption is that all of the endpoints would be evaluated in the AMRT, unless the conditions set forth in the guidelines for determining the selection of endpoints are met.

The AMRT involves exposure of maternal rats (designated F0 generation) from gestational day 6 (time of implantation), through parturition (birth), and through the lactation period until weaning of offspring (designated F1 generation) on post-natal day 21. F1 offspring (both sexes) are retained after weaning with no exposures for 10 weeks and then mated within groups. F1 males are necropsied after the mating. F1 females and their litters (designated the F2 generation) are retained until the F2 generation is weaned. F0 females (and a subset of F1 weanlings) are necropsied with organ weights and possible histopathology. F1 animals are evaluated for reproductive development (VP, PPS), estrous cyclicity, and, at necropsy, for organ weights, possible histopathology, andrological assessments, and T3/T4 (with TSH triggered). F2 weanlings are counted, sexed, weighed, examined externally, and discarded.

The AMRT differs from the ''standard'' 2-generation study design in that it:

a. Does not include exposures prior to mating, during mating, or during the early pre-implantation stage of pregnancy in the dams.

b. Does not include exposures to parental males.

c. Does not include direct exposure to the postweanling offspring; potential exposure is limited to *in utero* transplacental and/or lactational routes.

The AMRT differs from the 1-generation test (see Unit V.B.2.ii. of this notice) in that its study design provides for:

a. Exposure to the F0 dam only from gestational day 6 through weaning of the F1 offspring on post-natal day 21.

b. No exposure to parental males.

c. Mating of the F1 animals (who have not been directly exposed) to produce F2 offspring.

d. Following the F2 offspring to weaning (post-natal day 21).

ii. *1-Generation reproduction toxicity test*. A second alternative to the standard 2-generation reproductive toxicity test is a 1-generation reproductive toxicity test, which has been used in rats and mice. The 1-generation reproductive toxicity test has been used as a range-finding study prior to performance of a guideline 2-generation (or more) study for the last 10 years under EPA (TSCA/FIFRA) GLPs; the design is similar to that used by Sharpe et al. 1996. This is a shortened, scaled-down version of the new draft OPPTS and Final TSCA guidelines for reproductive toxicity testing. As with the 2-generation mammalian reproductive toxicity study, the default assumption is that all of the endpoints would be evaluated in the 1-generation test, unless the conditions set forth in the guidelines for determining the selection of endpoints are met.

The 1-generation test is a less comprehensive evaluation of functional reproductive development than the AMRT (since it does not follow F1 animals through production of F2 offspring), but it has the advantage of assessing post-natal development and adult reproductive capacity after *in utero* lactational and post-lactational exposure. In the presence of continued exposure, the post-natal component of the test is extended to evaluate acquisition of VP, PPS, estrous cyclicity, and andrological assessments in the F1 offspring. Inappropriate retention of Mullerian duct derivations (e.g., oviducts) in males and of Wolffian duct derivatives (e.g., seminal vesicles, epididymides) in females can be identified in all three proposed tests (with or without satellite F0 females and examination of term fetuses).

The 1-generation test involves a short prebreed-exposure period for male and female rats of the initial parental generation (designated F0), and exposure continues through mating, gestation, and lactation of F1 litters. F0 males are necropsied after F1 deliveries; F0 females are necropsied after F1 weaning. Postweanling F1 animals are directly exposed for a 10-week postwean period and are then necropsied. F1 animals are evaluated for reproductive development (VP, PPS), estrous cyclicity and at necropsy for organ weights, possible histopathology, andrological assessments, and T3/T4 (TSH triggered). F0 animals will undergo the same necropsy assessments.

The 1-generation test differs from the ''standard'' 2-generation study design in that it:

a. Is shorter (basic design calls for 2 weeks but it can be extended) than the standard 2-generation study (10 weeks to encompass one full spermatogenic cycle in rats), though it does include a prebreed-exposure period.

b. Does not evaluate effects of *in utero* and/or lactational exposure (and beyond) on generation of F2 offspring though it does include direct exposure of F1 offspring after weaning, including exposure through puberty and sexual maturation. F1 male and female reproductive organs (weight/histology), estrous cyclicity, and andrological endpoints are assessed at scheduled necropsy on post-natal day 90 ± 2.

The 1-generation test differs from the AMRT in that its study design provides for:

a. Exposure to both male and female F0 parental animals prior to mating, during mating, and during gestation and lactation of F1 offspring (F0 males are necropsied after F1 deliveries, F0 females are necropsied after F1 weaning).

b. Direct exposure of postweanling F1 offspring after lactation until termination.

c. No mating of F1 animals to produce F2 offspring.

C. Route of Administration

As part of the test guideline, EPA will provide guidance on a route of administration for each screen and test. Tier 1 screening assays may employ dosing routes that maximize the likelihood of detecting endocrine activity such as ip. Conversely, Tier 2 tests will employ routes of administration based upon the most ecologically relevant exposure pathway to provide data relevant for risk assessment.

The route of administration for the uterotrophic assay is sc injection while the route for the modified uterotrophic assay and 14-day intact adult male assay with thyroid is an ip injection. The route for all other mammalian *in vivo* assays is gavage (orogastric intubation). The parenteral (non-oral) routes avoid the first-pass metabolic effect of the liver and will permit detection of potential endocrine disruptors that are active as parent compounds and which undergo significant first-pass metabolism. Hepatic xenobiotic metabolism does occur eventually after parenteral administration (substantially with ip), so the potential effects of metabolites will be evaluated as well by these routes. Compounds are occasionally metabolized by the gut microflora; this type of metabolism has been shown to be important for some plant-derived estrogens. The oral route of exposure will allow for this type of metabolism.

VI. Implementation

This section of the **Federal Register** notice discusses the implementation steps for the EDSP and many of the issues EPA must deal with in its implementation.

A. Overview of Implementation Steps and Timeline

There are many elements associated with the development and implementation of the EDSP. A timeline that shows the key elements and their relationship to each other is provided in Figure 2.

They include:

Implementation steps	Estimated completion dates
EDSTAC Final Report and Recommendations	Completed
Development of EPA's EDSP	Completed
Public comment on EPA's EDSP	February 26, 1999
SAB/SAP Peer Review Processes	April 1, 1999
HTPS Demonstration	February 1999
HTPS	June 2000
EDPSD	June 2000
Priority Setting for Tier 1 Phase 1	November 2000
Tier 1 Standardization and Validation September	2001
Tier 1, Phase 1 TSCA Test Rule Notice of Proposed Rulemaking (NPRM) and FQPA Orders	December 2001
Tier 1, Phase 1 TSCA Final Test Rule	June 2003

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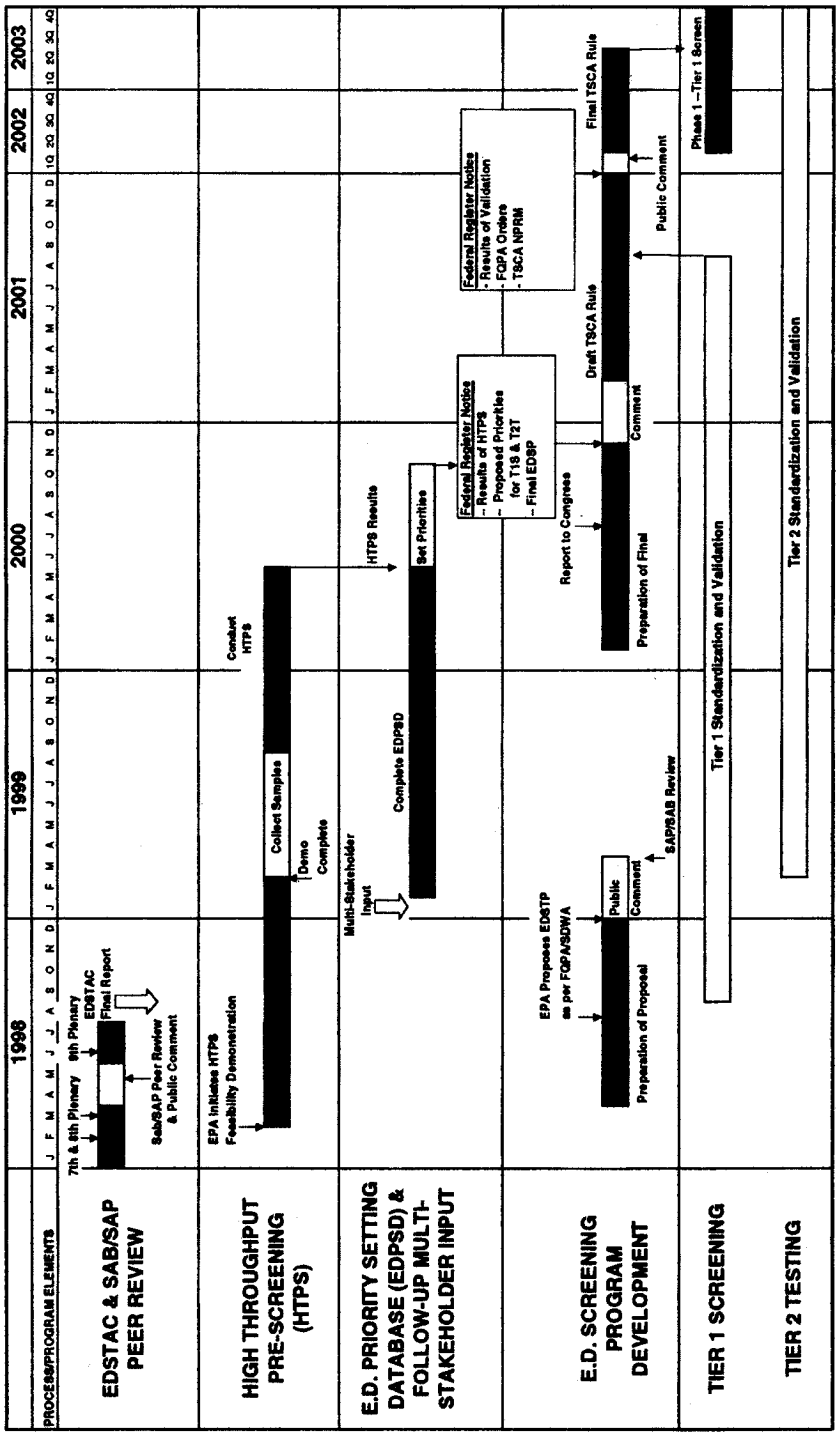
FIGURE 2-TIMELINE FOR IMPLEMENTATION OF THE EDSP



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As noted, the recommendations of EDSTAC form the basis for EPA's endocrine-disruptor screening and testing strategy. Today, EPA is soliciting comments on its strategy for screening and testing substances for their potential to disrupt the EAT. These comments and the Agency's proposal will be reviewed by a joint meeting of the EPA SAB and FIFRA SAP in March 1999. Notice of the meeting site and specific times will be published in the **Federal Register**.

EPA plans to begin running chemicals through the HTPS in August 1999.

The Agency will submit a report to Congress and plans to issue a notice in the **Federal Register** in the year 2000 adopting final policies for the screening program based on comments of the SAP/SAB and the comments received in response to this notice. The year 2000 notice will also propose the *Priority List* of chemicals and mixtures for Tier 1 screening. The proposed screening *Priority List* will be based on information in the EDPSD including the results of the HTPS. EPA may also issue a procedural rule that describes the procedures related to implementation of the EDSP.

EPA plans to publish the results of the standardization and validation effort for the screening battery along with guidelines for the screening assays that flow from this effort in the **Federal Register** in 2001. The standardization and validation of Tier 2 tests will be undertaken approximately in parallel with that of the screening battery. However, the test validation program is anticipated to take longer than the screening validation program because the Tier 2 tests take much longer to run than the Tier 1 screening assays.

In late 2001, EPA plans to issue testing orders to the first group of pesticides and other chemical substances that are subject to the authority provided to EPA under the FFDCA and SDWA. In parallel to these activities, EPA may propose a TSCA test rule to require screening of chemicals that may not be covered by the FFDCA/SDWA. EPA could propose the TSCA test rule in 2001 and promulgate it in mid 2003. The screening program will operate in phases so as to not overwhelm resources. The number of phases and length of time between phases will depend on available resources and the number of chemicals proposed for screening in each phase. EPA plans to review its initial prioritization of chemicals and issue a separate proposed rule for each screening phase. This would allow the results from the first phase of screening to improve the priority setting for the second phase of screening.

Tier 2 testing of chemicals that are part of the first phase of Tier 1 screening would begin after review of screening data indicated that testing was warranted. Standardization and validation of Tier 2 tests will take from 2 to 5 years. EPA plans to require tests as soon as they are available and not wait for the full battery to initiate Tier 2 testing. Orders under FFDCA, FIFRA, or SDWA would be issued on individual chemicals as their review is completed. TSCA rules would be issued for a group of chemicals, probably on an annual basis.

B. HTPS Demonstration

EPA has initiated a demonstration program to validate use of HTPS technology to screen chemical substances for EAT disrupting properties. The demonstration program is projected to be completed in February 1999. If EPA successfully validates HTPS through the demonstration program, it could begin running chemical substances through HTPS in August of 1999.

C. HTPS Priority-Setting Project

After completion of the HTPS demonstration and validation project, EPA plans to conduct the HTPS on approximately 15,000 chemicals (commercial chemicals produced in amounts greater or equal to 10,000 pounds per year and all pesticides) to supplement existing information. EPA will fund the actual screening of these compounds and is soliciting industry cooperation in supplying samples of pesticides and commercially produced chemicals. One major issue in HTPS is how to deal with the need for analytical characterization of so many chemicals. The cost of chemical analysis is more than an order of magnitude greater than the cost of the HTPS battery.

Option One is to require full analysis on each chemical prior to HTPS. This is the usual requirement for toxicological testing.

Option Two is to perform chemical analysis after HTPS on those substances that test positive.

Option Three is to rely on the chemical identity and composition claims of the chemical supplier.

EPA favors Option Two as a cost effective alternative to full analysis of every chemical. Nevertheless, every sample submitted to EPA should be accompanied by some information regarding its analytical characterization. It should at a minimum state whether the material is a technical grade, analytical grade, etc., to what extent it has been characterized, and note the concentration or percentage of the sample comprised by the test substance.

EPA plans to subject chemicals to HTPS that will bypass Tier 1 screening as well as those that need screening. The rationale for conducting HTPS on these chemicals is:

1. Data generated from the HTPS assays will be valuable for receptor-binding mechanisms even though such data by itself cannot be used to determine whether or not a chemical may be an endocrine disruptor.

2. As an ancillary benefit, the data can be used to improve and validate QSAR models.

3. For food-use pesticides that will probably undergo reregistration and tolerance reassessments prior to the availability of validated Tier 2 tests, HTPS data can be used along with other relevant testing information to help determine if and when they should undergo any additional endocrine-disruptor testing.

D. Priority-Setting Data Base (EDPSD) Development

As described in Unit IV.C. of this notice, EPA plans to use existing exposure, effects and statutory-related data and information to sort and prioritize chemicals for endocrine-disruptor screening and testing. To maximize its resources, EPA will rely upon data excerpted in electronic format instead of primary literature. Recognizing the numerous data bases of potential utility to initial sorting priority setting (see Appendix H of the EDSTAC Final Report), EPA plans to assemble the relevant and useful data sources into a single-relational data base. Development of this data base was initiated by the EDSTAC but not completed due to time and resource constraints of the EDSTAC process. EPA has resumed efforts to complete development of the prototype EDPSD initiated by EDSTAC. EPA is publishing elsewhere in this issue of the **Federal Register** a document announcing a priority-setting workshop for multi-stakeholders and the use of the EDPSD during the comment period.

The purpose of the workshop is to provide stakeholders an opportunity for input into the design and implementation of the priority-setting system. The focus of the workshop is to discuss the basic structure and functioning of the priority-setting system. Specifically, the workshop will address the definition of compartments, principles and approaches for developing rankings within compartments, and for assigning overall

weighting factors to the various compartments and categories.

E. Process for Public Nominations for Chemical Screening

Chemical nominations from the public were considered to be an important part of the nominations process by EDSTAC because they provide a mechanism to identify and screen chemicals which may result in high exposures in local communities but which do not receive national attention. EPA proposes to establish a nomination process. The nominations process could be a formal petition process or an informal one such as a letter submitted to the Agency. EPA belives that any nomination should be signed and should include the following information:

Statement that it is nominating a chemical for screening in the EDSP, identification of the chemical.

Statement of the reasons for its nomination.

Although EPA does not believe it can legally protect the identity of nominators, employees in the chemical industry are protected by law against reprisals from employers for reporting a chemical under TSCA (15 U.S.C. 2622) and any threats or reprisal of any kind should be reported to the U.S. Secretary of Labor with a copy of the threat or reprisal report to the EPA Administrator.

F. Standardization and Validation of Assays, Screening Battery, and Tests

Validation is the scientific process by which the reliability and relevance of an assay method are evaluated for the purpose of supporting a specific use (ICCVAM, 1997). Relevance refers to the ability of the assay to measure the biological effect of interest. Measures of relevance can include sensitivity (the ability to detect positive effects), specificity (the ability to give negative results for chemicals that do not cause the effect of interest), statistically derived correlation coefficients, and determination of the mechanism of the assay response with the toxic effects of interest. Reliability is an objective measure of a method's intra- and inter-laboratory reproducibility. The process of validation includes standardization, that is, definition of conditions under which the assay is run (species, strain, culture medium, dosing regimen, etc.). Standardization is critical to ensure reliability, that is, valid, consistent results between laboratories.

FFDCA as amended by the FQPA requires EPA to ''develop a screening program, using appropriate validated test systems and other scientifically relevant information, to determine whether certain substances may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or such other endocrine effect as the Administrator shall designate.''

EPA convened a meeting of the Domestic Validation Task Force (Task Force) comprised of experts and representatives of major stakeholders on August 6, 1998, and is scheduled to meet on a bimonthly basis during 1999. The Task Force is made up of members from Federal agencies, industry, and public interest groups. The purpose of the Task Force is to implement the validation program for the screens and tests. In March 1998 and November 1998, the OECD Endocrine Disruptor Testing and Assessment Workgroup met to initiate an international validation program for endocrine-disruptor screening and testing. The international validation program is important in developing an internationally harmonized approach to endocrine-disruptor screening and testing. An internationally harmonized approach saves money by reducing duplicative testing. EPA anticipates that some, but by no means all, of the assays it is proposing will be included in the international validation program. The majority of the screening assays and the screening battery itself will have to be validated in the domestic validation program.

Standard protocols for most of the screening assays and tests are now being developed. Most of these should be ready for Task Force review and approval in 1999. EPA is inviting laboratories to participate in the validation program. Laboratories that are interested in the participating in any aspect of the validation program should contact Anthony Maciorowski (see the ''FOR FURTHER INFORMATION CONTACT'' section of this notice). Participating laboratories will receive a standard protocol for each assay they want to conduct and appropriate control and test chemicals from the EPA or its agent. EPA is planning to begin the laboratory phase in the spring of 1999. Some assays which need further development will not begin validation until late 1999 or the year 2000.

G. Implementation Mechanisms

As stated previously, EPA believes that the FFDCA and SDWA provide authority to require the testing of many of the approximately 87,000 chemical substance that it wishes to test. As appropriate, EPA also will use other testing authorities, such as those under FIFRA and TSCA. Likewise, to the extent that EPA is concerned about the endocrine disrupting potential of other chemical substances, it will work with other Federal agencies and departments to ensure that these substances also are tested. EPA will determine under which authority it will require testing of specific chemicals on a case-by-case basis. A brief description of EPA's major testing authorities and guidance on their application to the EDSP are set forth in this unit.

1. *FFDCA testing authority*. Under the FFDCA, as amended by FQPA, EPA has authority to order registrants, manufactures, or importers to test certain chemical substances, including pesticide chemicals and any other substance that may have an effect that is cumulative to an effect of a pesticide chemical if EPA determines that a substantial population may be exposed to such substances.

Under the FFDCA, ''pesticide chemical'' includes ''any substance that is a pesticide within the meaning of FIFRA, including all active and inert ingredients.'' It also includes impurities (see 40 CFR 177.81). The testing requirement is not restricted to pesticides used on foods.

EPA is still working out how to determine whether a substance ''may have an effect that is cumulative to the effect of a pesticide chemical.'' However, at a minimum, EPA believes that if the mechanism of action of a pesticide chemical and a nonpesticide chemical is the same, their effects are additive and therefore may be cumulative. Likewise, when the metabolic detoxification or clearance process of a pesticide chemical and a nonpesticide chemical are the same, exposure to the nonpesticide chemical may slow the clearance of the pesticide, and therefore, increase the pesticide chemical's toxicity. This is an example of a cumulative effect even when the two chemicals do not operate by the same mechanism of toxicity or cause the same toxic effect. The same argument would also apply to enzyme poisons or noncompetitive inhibitors of pesticide metabolism that slow or completely block the metabolic pathway of a pesticide. EPA is interested in receiving comment on these and other examples or on methods to determine whether a substance may have an effect that is cumulative to the effect of a pesticide chemical.

The phrase ''substantial population'' is used in FFDCA section 408(p)(3)(B) and in SDWA section 1457 but is not defined in either of these statutes. Based upon EPA's experience under TSCA, it is necessary for the Agency to define this term. Under TSCA section 4(a)(1)(B) EPA defined ''substantial human

exposure'' in terms of numbers of persons exposed based on a sliding scale that reflected that more direct exposures would require smaller numbers of persons exposed in order to be substantial than less direct exposures would (58 FR 28736, May 14, 1993). EPA is offering no definition of ''substantial population'' for SDWA and FIFRA purposes at this time but seeks public comment on an appropriate definition.

2. *SDWA testing authority*. Congress amended SDWA to give 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).

Drinking water contaminants may include, but may not be limited to, pesticide active and inert ingredients and their degradates, commercial chemicals and their degradation products, substances formerly manufactured and used as pesticides or commercial chemicals (orphan chemicals), or natural substances.

3. *FIFRA testing authority*. FIFRA section 3(c)(2)(B) provides EPA authority to require pesticide registrants to submit to EPA additional data regarding a pesticide if EPA determines that the additional data are required to maintain in effect an existing pesticide registration. Under this provision, EPA could require submission of endocrine effects data for registered pesticides and for chemicals that may have an effect that is cumulative to that of a pesticide. FIFRA sections 3(c)(2)(A), 3(c)(5), 3(c)(7), and 3(d) also give EPA authority to require testing.

4. *TSCA testing authority*. TSCA section 4 provides EPA with authority to require testing of certain chemical substances, not including pesticides or food additives among other things, if the Agency finds that the chemical substance or mixture:

i. May present an unreasonable risk of injury to health or the environment.

ii. There are insufficient data and experience from which the Agency can determine the effects of such substance or mixture on health or the environment.

iii. Testing with respect to such substance or mixture with respect to such effects is necessary to develop such data.

Alternatively, EPA can require testing if the Agency finds that:

i. A chemical substance or mixture is or will be produced in substantial quantities and:

a. It enters or may reasonably be anticipated to enter the environment in substantial quantities, or

b. There is or may be significant or substantial human exposure to such substance or mixture.

ii. There are insufficient data and experience which from which the Agency can determine the effects of such substance or mixture on health or the environment.

iii. Testing with respect to such substance or mixture with respect to such effects is necessary to develop such data.

EPA achieves TSCA testing through rulemaking and enforceable consent agreements (ECAs). For more information on EPA's TSCA testing authority see 40 CFR part 790.

Some chemicals might be subject to more than one testing authority. Inert pesticide ingredients will frequently have TSCA uses in addition to their use as inert ingredients in pesticide formulations and could be screened or tested under TSCA or FFDCA/FIFRA authorities. TSCA chemicals found in drinking water sources could also be screened or tested under SDWA or TSCA. Compared with order authority under FIFRA, FFDCA, or SDWA, a test rule is a slow and labor intensive mechanism. Therefore, the Agency believes that when a choice is possible it is in the public interest to require screening and testing under its FIFRA, FFDCA, or SDWA authorities, rather than under TSCA, when it has that option.

H. Data Compensation Issues

The FFDCA, as amended, requires EPA ''to the extent practicable,'' to ''minimize duplicative testing of the same substance for the same endocrine effect, [and] develop, as appropriate, procedures for fair and equitable sharing of test costs.''

To meet these requirements, EPA is planning to adopt procedures similar, but not identical, to both TSCA's and FIFRA's data compensation procedures. If EPA knows that there is more than one registrant, manufacturer, and/or importer of a specific chemical, it will order each to test the chemical. As part of the order, it will include a list of all of the parties who receive equivalent orders and require the parties to work together to minimize duplicative testing and share testing costs. The parties may notify EPA of other parties not listed who also manufacture or import the chemical. Alternatively, or in addition, EPA will publish the order in the **Federal Register** and require parties not listed to self identify. If the parties are unable to work out testing and data compensation responsibilities, they will be required to submit to binding arbitration. If a party fails to comply with an arbitrator's decision, it will be subject to the penalties described in FFDCA section 408(p)(5)(C).

If, after completion of the testing, another party seeks to use the resulting data in support of a pesticide registration, it will be required to comply with FIFRA sections 3(c)(1)(F) or 3(c)(2)(B) which require compensation for data. Likewise, TSCA requires parties to compensate test sponsors if they manufacture or import a substance covered by a test rule within 5 years of the submission of the last required study. Chemicals being tested pursuant to a rulemaking under TSCA will follow the TSCA procedures for reimbursement under 40 CFR part 791.

I. Data Submission and Collection

EPA is proposing to post an electronic form for the capture of data from screening and testing so that these data can be easily uploaded into the Endocrine Knowledge Base (EKB) being developed by the FDA's National Center for Toxicological Research. The EKB will be the repository of all data from the EDSP as well as other sources of endocrine effects testing and research. The data base will thus serve research and regulatory purposes. As the data base is further developed, EPA will provide guidance on how to submit data electronically to be compatible with the EKB.

J. Data Release and CBI

FFDCA section 408(p)(5)(B) requires that EPA, to the extent practicable, develop, as necessary, procedures for handling CBI submitted as part of the EDSP. EPA anticipates that much of the information that registrants and manufacturers submit under the auspices of its EDSP will be health and safety information that generally does not warrant CBI protection. Nevertheless, EPA is interested in receiving comments from potential data submitters concerning whether they think any of the information will deserve CBI protection. If data submitters believe that certain information will be deserving of protection, the Agency is interested in receiving comments on the specific types of information that might need protection and on procedures that the Agency could develop to verify the validity of CBI claims and to ensure protection of valid CBI. EPA also is interested in receiving comments on whether current procedures under FIFRA and TSCA would be adequate and, if so, how they should be applied.

EPA is considering adopting FIFRA CBI procedures for data submitted on pesticide active ingredients and TSCA CBI procedures for all other substances. If necessary, EPA will develop additional procedures to ensure that any valid confidential business information is protected from disclosure.

K. Reporting Requirements Under TSCA 8(e) and FIFRA 6(a)(2)

The following provides EPA's guidance on the reporting obligations under the TSCA section 8(e) and FIFRA section 6(a)(2) with respect to results from certain priority-setting studies and *in vitro* screening assays that industry or others may conduct voluntarily or as part of EPA's EDSP. TSCA section 8(e) requires that ''[a]ny person who manufactures, processes, or distributes in commerce a chemical substance or mixture and who obtains information which reasonably supports the conclusion that such substance or mixture presents a substantial risk of injury to health or the environment shall immediately inform [EPA] of such information'' (15 U.S.C. 2607(e)). Likewise, FIFRA section 6(a)(2) requires registrants that, after registration of a pesticide, have additional factual information regarding unreasonable adverse effects on the environment of the pesticide to submit the information to EPA (7 U.S.C. 136d(a)(2)).

EPA will likely adopt as part of its EDSP both *in vitro* and *in vivo* assays that assess selected hormonal endpoints. Based on the current state of the science, EPA considers the results of endocrine disruptor *in vitro* screening assays to be indicators of potential endocrine activity. Whether performed at the bench or in a high throughput mode, results from *in vitro* assays may suggest some mechanisms of endocrine activity (e.g., hormone receptor binding, binding plus transcription, cell proliferation, steroidogenesis, etc.). Thus, the results of these *in vitro* assays are arguably within the scope of TSCA section 8(e) and FIFRA section 6(a)(2). At this time, however, EPA can not conclude that the results of these *in vitro* assays translate into an understanding of particular health or environmental hazards and risks *in vivo*. Therefore, based on the current state of the knowledge, EPA will not, at this time, require submission of TSCA section 8(e) or FIFRA section 6(a)(2) reports containing only the results of these *in vitro* assays. Registrants, manufactures, or importers are, nevertheless, encouraged to submit the data voluntarily. If these test results are included with other information reportable under TSCA section 8(e) or FIFRA section 6(a)(2), then they must be reported.

L. Exemptions

There are several circumstances in which exemptions from screening or testing requirements are appropriate. The FFDCA section 408(p) provides for exemptions from its requirements if EPA determines that a substance is anticipated not to produce any effect in humans similar to an effect produced by a naturally occurring estrogen. Although EPA has not determined when or under what circumstances it will grant exemptions from FFDCA 408(p) requirements, examples of the types of chemicals that might warrant such exemptions include class 4 pesticide formulation inerts—those inert ingredients in pesticide formulations judged by EPA to be virtually non-toxic (for example cookie crumbs)—and strong mineral acids and strong mineral bases, which would likely interact with tissue at the portal of entry giving rise to localized lesions rather than systemic effects. The strong reactivity of these substances would cause interaction with membranes and other biological chemicals before the chemical reached the endocrine receptors.

EPA is considering establishing a petition process as a means of establishing exemptions from screening. The details of this process could be set forth in the procedural rule EPA is considering issuing for the EDSP. EPA is asking for comments on criteria that might form the basis for granting exemptions.

Exemptions under FFDCA 408(p) are not the same as exemptions under FFDCA section 408(c). Please note also that the term exemption as used under FFDCA section 408(p) is different from, and should not be confused with, the use of this term under TSCA section 4(c). An exemption under FFDCA section 408(p) means that testing requirements do not apply. However, under TSCA section 4(c) an exemption is a mechanism for avoiding duplicative testing. Under TSCA section 4(c) an exemption can be granted when data are being or have been generated by a responsible party and, therefore, other responsible parties can reimburse the test sponsor for a portion of the cost. A similar cost sharing provision exists for data compensation among registrants under FIFRA (see Unit VI.H. of this notice). Unless otherwise indicated, the term exemption used in this notice will be used in the sense in which it is used under FFDCA section 408(p), that is, a waiver of all testing obligations.

M. Use of Significant New Use Rules (SNURs) Under TSCA

During the EDSTAC deliberations, concern was expressed that under certain circumstances less than the full Tier 2 testing would be permitted on chemicals based on their limited use and exposure profile. For instance, a pesticide registered for contained use only may result in human exposure but negligible or no environmental exposure. Therefore, performing the 2-generation mammalian reproductive effects test may be all that is needed to assess the hazards of this substance. Granting permission to limit Tier 2 testing does not present a problem for pesticides because pesticide registration limits the uses of the pesticide to those contained in the registration application. If a pesticide registrant wants to expand the uses and therefore potentially the exposure to a pesticide, the registrant must apply to register the expanded uses. The same is not true for chemicals under TSCA, since TSCA is not a registration statute. Once a commercial chemical is on the market it can ordinarily be used freely for any purpose resulting in exposures that were not occurring at the time testing requirements were promulgated. A potential solution to this dilemma lies in EPA's authority under TSCA section 5(a)(2) to issue SNURs.

A SNUR defines certain uses of a chemical as new uses. Before a manufacturer or processor can use a chemical for one of the defined new uses, the manufacturer or processor must notify EPA of such intention at least 90 days before commencement of the new use. A SNUR thus subjects an existing chemical that triggers a new use to the same review that a new chemical receives. Submission and review of the new use can be tied to the performance of testing and submission of test data to EPA if there is a test rule that covers that chemical.

EPA is considering the development of a SNUR based on a manufacturer's showing of limited use and exposure as a condition for granting a waiver for limited Tier 2 testing for TSCA chemicals (i.e., permission to perform fewer than the five tests in Tier 2 based upon exposure considerations). If the manufacturer's claims for limited use and exposure are refuted in the significant new use rulemaking process by someone who is already using the chemical in such a manner, the SNUR will not be valid and the manufacturer will be required to perform the full battery of Tier 2 tests required in the test rule issued for that chemical under the EDSP.

N. Relationship Between the EDSP and Related Actions Under TSCA

Several other testing actions under TSCA may affect chemicals in the EDSP. Actions planned or underway include the Hazardous Air Pollutants (HAPs) test rule (61 FR 33178, June 26, 1996) (FRL–4869–1) as amended, the Children's' Health test rule, the Agency for Toxic Substances and Disease Registry (ATSDR) test rule, the High Production Volume (HPV) testing initiative and the Screening Information Data Set (SIDS) Program on HPV chemicals. None of the EDSP Tier 1 screening assays is being considered for by these actions. The SIDS and HPV testing programs do not meet either the screening or testing requirements of the EDSP. The only likely overlap in testing requirements is the 2-generation mammalian test, which is proposed in the HAPs rule and being considered in the Children's Health test rule and ATSDR test rule. The reproductive effects testing for these programs will meet the Tier 2 mammalian reproductive effects testing requirement for the EDSP if the 1998 or later guideline for a 2-generation mammalian reproductive effects study is used. The results from some of these testing programs likely will be available before final testing decisions are made under the EDSP. It is possible that if the results of the 2-generation test (with endocrine-sensitive endpoints including thyroid) generated under one of these other testing programs is negative that only the fish gonadal recrudescence assay would need to be performed to satisfy the testing requirements of the EDSP. The correlation of various test results in the validation study will provide more information on which to make this judgment. If the mammalian 2-generation test were positive, the other Tier 2 tests would have to be run depending upon the exposure profile of the chemical in question.

O. Analysis of Data in the EDSP

EPA discussed use of HTPS data for priority setting for Tier 1 screening and as part of the weight of evidence consideration to determine when a chemical should be tested in Tier 2. These data may also used in conjunction with other data to help determine if adverse effects observed in Tier 2 are due to endocrine disruption or from another cause. The Tier 1 data will also serve a dual purpose. They will be used to make the determination of which chemicals receive Tier 2 testing and will also be used to help interpret positive results observed in Tier 2 testing.

More detailed guidance regarding the assessment of hazards due to endocrine disruption must await both the results of the standardization and validation program and ongoing research. EPA intends to review the need for revising its standard evaluation procedures for interpreting studies and its human health and ecological risk assessment guidelines as relevant data from these programs become available.

VII. Issues for Comment

1. The FFDCA, as amended, requires EPA to screen pesticides for estrogenic effects that may affect human health. EPA has decided that it is scientifically appropriate to focus on EAT effects, not just estrogenic effects. Is this an appropriate scope for the EDSP?

2. Are there classes of chemicals besides the ones identified in Unit VI.L. of this notice that should be exempted (excluded) from the EDSP? What criteria and what burden of proof should be applied to claims of persons seeking to exempt chemicals from screening? What type of process should EPA establish?

3. As discussed in Unit IV.E. of this notice, EPA is proposing a compartment-based (or set-based) approach to priority setting as a way of accommodating the real world situation of uneven data. Under the compartment-based approach, EPA will group the chemicals into sets based on the existence of factual information in a given area. Thus, priority ranking can be made fairly among chemicals, i.e., chemicals will compete for priority with other chemicals on the basis of comparable data and will not be assigned lower priority for lack of information. Are these principles and the compartment-based approach to priority setting reasonable? Are there alternatives to the compartment-based approach which EPA should consider?

4. As recommended by EDSTAC, EPA is proposing that polymers with an average number molecular weight greater than 1,000 daltons be excluded from priority setting and screening unless they are pesticide chemicals or unless their monomers, oligomers, or leachable components are shown to have endocrine-disrupting potential in Tier 1 screening. Is this approach scientifically sound?

5. EPA is developing a relational data base to assist in setting priorities for screening. The relational data base is intended to import existing data and information and allow its synthesis, as well as the estimation of certain parameters through modeling. EPA and EDSTAC consider the relational data base to have great value in helping to identify the specific compartments under the compartment-based priority-setting approach. The data base will also be helpful in selecting chemicals for the first and subsequent rounds of screening. The data fields currently in the data base are defined in Chapter 4 of the EDSTAC Final Report. What additional data fields or types of data should EPA include as it further develops the relational data base?

6. EPA is soliciting industry's cooperation in supplying chemicals for the HTPS. Is this an appropriate role for industry and is industry willing to do so?

7. EPA plans to screen and, if appropriate, test representative mixtures to which large or identifiable segments of the population are exposed. The high-priority mixture categories include: Chemicals in breast milk, phytoestrogens in soy-based infant formulas, mixtures commonly found at Superfund sites, common pesticide/fertilizer mixtures found in ground and surface water, disinfection byproducts, and gasoline. EPA plans to screen and test one representative mixture from each category.

a. Can standardized representative mixtures be developed? If so, how should the chemical combinations, ratios, and doses be selected for mixtures?

b. Is the proposal a reasonable way to address the practicality of screening and testing mixtures?

c. Are the six categories of mixtures the most appropriate to address first?

d. Are there other mixture categories that should be included in addition to, or instead of those identified (e.g., Should fish tissue contaminants be one of the first mixtures)?

e. If a mixture is positive in Tier 1, should the whole mixture be tested in Tier 2 or should EPA attempt to identify the active component(s) and test it (them) in Tier 2?

8. EPA has identified a screening battery consisting of *in vitro* and *in vivo* assays to address EAT effects. Will the battery, once validated, be capable of detecting such effects in a consistent and reliable manner?

9. EPA is planning to require that the Tier 1 screening *in vivo* assays be conducted at one dose, with appropriate use of range finding studies and other information (i.e., HTPS results) to inform dose selection. The single-dose approach was adopted to save testing resources. The SAB/SAP in a preliminary consultation raised concern about relying on only one dose level and suggested that EPA require a minimum of two doses and preferably three to ensure that tests did not result in false negatives. Does the potential risk of

false negatives outweigh the cost savings of running the Tier 1 screening *in vivo* assays with only one dose?

10. EDSTAC could not identify existing practical vertebrate endocrine disruptor screening assays that incorporated exposure *in utero* or *in ovo*. Do such screening assays exist?

11. Is adequate coverage of the thyroid provided in the recommended Tier 1 screening battery? Does the Tier 1 screening battery provide adequate coverage of non-receptor mediated pathways?

12. EPA is proposing a Tier 2 testing battery to delineate dose-response relationships of chemicals that yield positive results in the screening battery. Do the tests provide sufficient rigor to identify adverse effects and establish dose response for disruption of the EAT?

13. Will the Tier 2 tests be adequate to detect all known EAT endpoints in chemicals that bypass Tier 1 screening?

14. Tier 2 tests will identify the adverse effects due to endocrine disruption as well as reproductive and developmental effects caused by non-endocrine mechanisms of toxicity. Thus, it may not be possible to determine that a substance is an endocrine disruptor if it bypasses tier 1 screening. Is it important to be able to identify substances as endocrine disruptors from the standpoint of conducting a hazard assessment?

15. If the results of the 2-generation test (with endocrine-sensitive endpoints including thyroid) generated under one of these other testing programs is negative what additional screening or testing should be required to demonstrate that the chemical is not an endocrine disruptor?

16. FFDCA gives EPA authority to test pesticides and substances ''that are cumulative to the effect of a pesticide.'' EPA is interested in receiving comment on how the term ''cumulative to the effect of a pesticide'' should be applied in defining additional substances which can be tested under FFDCA.

17. How should EPA define substantial population as used in FFDCA section 408(p) and SDWA section 1457?

8. Is EPA's proposal to adopt FIFRA cost sharing provisions for data received under FIFRA and TSCA cost sharing provisions for all other substances feasible and practical?

19. Is EPA's proposal to adopt FIFRA CBI procedures for active pesticide ingredients and TSCA CBI procedures for all other substances feasible and practical? TSCA makes health and safety data freely available. The chemical portion of chemical substances comprising formulated products is confidential under both statutes.

20. Should EPA permit chemicals to receive less than the full Tier 2 testing battery under certain circumstances? Should EPA issue a SNUR for TSCA chemicals that are subject to limited Tier 2 testing?

21. Should EPA issue a procedural rule codifying many of the procedures discussed in Unit VII. of this notice?

VIII. References

The Agency's actions are supported by the references listed in this unit and cited in this notice. These references are available in the public record for this notice under docket control number OPPTS–42208 in the TSCA Docket, see the ''ADDRESSES''section in this notice.

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IX. Public Record and Electronic Submissions

The official record for this notice, as well as the public version, has been established for this notice under docket control number OPPTS–42208 (including comments and data submitted electronically as described in this unit). A public version of this record, including printed, paper versions of electronic comments, which does not include any information claimed as CBI, is available for inspection from 12 noon to 4 p.m., Monday through Friday, excluding legal holidays. The official record is located at the address in Unit I.B.3. of this notice.

Electronic comments can be sent directly to EPA at:

oppt-ncic@epa.gov.

Electronic comments must be submitted as an ASCII file avoiding the use of special characters and any form of encryption. Comment and data will alsobe accepted on disks in Wordperfect 5.1/6.1 or ASCII file format. All comments and data in electronic form must be identified by the docket control number OPPTS–42208. Electronic comments on this notice may be filed online at many Federal Depository Libraries.

List of Subjects

Environmental protection, Chemicals, Drinking water, Endocrine disruptors, Hazardous substances, Health and safety, Pesticides and pests.

Authority: 21 U.S.C. 346a(p); 42 U.S.C. 300j–17; 7 U.S.C. 136a; 15 U.S.C. 2604.

Dated: December 21, 1998.

Lynn R. Goldman,

Assistant Administrator for Prevention, Pesticides and Toxic Substances.

[FR Doc. 98–34298 Filed 12–23–98; 9:49 am]

BILLING CODE 6560–50–F

ENVIRONMENTAL PROTECTION AGENCY

[OPPTS–42207; FRL–6052–8]

Endocrine Disruptor Screening Program; Priority-Setting Workshop

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice.

SUMMARY: This notice invites public participation in a workshop to discuss the development of a priority-setting system for the selection of chemicals for testing in the Endocrine Disruptor Screening Program (EDSP). The recommendations of the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) and the Agency's subsequent Statement of Policy contain a set of principles and a general strategy for setting priorities for testing. The Agency is now commencing the detailed design phase of the priority-setting system and seeks public input on the design of the system.

DATES: The workshop will be held on Wednesday, January 20, 1999, from 10 a.m. to 5 p.m. and Thursday, January 21, 1999, from 9 a.m. to 4 p.m. Comments may be submitted during the workshop or after the workshop until February 22, 1999.

ADDRESSES: The workshop will be held at the Crystal City Marriott Hotel, 1999 Jefferson Davis Hwy., Arlington, VA; telephone (703) 413–5500, toll-free reservation line (800) 228–9290.

Comments should be sent to Patrick Kennedy or James Darr and to the OPPTS Document Control Officer. Comments may be sent electronically or by mail to: Patrick Kennedy, e-mail address: kennedy.patrick@epa.gov or Jim Darr, e-mail address: darr.james@epa.gov; Office of Pollution Prevention and Toxics (7406), Environmental Protection Agency, 401 M St., SW., Washington, DC 20460.

Each comment must bear the docket control number OPPTS–42207. All comments should be sent in triplicate to: OPPT Document Control Officer (7407), Office of Pollution Prevention and Toxics, Environmental Protection Agency, 401 M St., SW., Room G–099, East Tower, Washington, DC 20460.

Comments and data may also be submitted electronically to: oppt.ncic@epa.gov. Follow the instructions under Unit V. of this notice. No Confidential Business Information (CBI) should be submitted through e-mail.

All comments which contain information claimed as CBI must be clearly marked as such. Three sanitized copies of any comments containing information claimed as CBI must also be submitted and will be placed in the public record for this rulemaking. Persons submitting information on any portion of which they believe is entitled to treatment as CBI by EPA must assert a business confidentiality claim in accordance with 40 CFR 2.203(b) for each such portion. This claim must be made at the time that the information is submitted to EPA. If a submitter does not assert a confidentiality claim at the time of submission, EPA will consider this as a waiver of any confidentiality claim and the information may be made available to the public by EPA without further notice to the submitter.

FOR FURTHER INFORMATION CONTACT: For information related specifically to the workshop: Patrick Kennedy, telephone: (202) 260–3916, e-mail address: kennedy.patrick@epa.gov or Jim Darr, telephone: (202) 260–3441, e-mail address: darr.james@epa.gov; Office of Pollution Prevention and Toxics (7406), Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. For general information or copies of the ESTAC Report: TSCA Hotline, Environmental Assistance Division (7408), Office of Pollution Prevention and Toxics, Environmental Protection Agency, 401 M St., SW., Washington, DC 20460; telephone (202) 554–1404, TDD (202) 554–0551; e-mail address: TSCA-Hotline@epa.gov.

SUPPLEMENTARY INFORMATION:

I. Background

The Agency first set forth the basic components of the EDSP in an August 11, 1998 (63 FR 42852) (FRL–6021–3) **Federal Register** notice. A more detailed Statement of Policy has been developed and is published elsewhere in this issue of the **Federal Register**.

The EDSP has five major components:

1. Sorting, in which chemicals are classified according to the availability of information on each chemical's endocrine-disrupting potential.

2. Priority setting, in which EPA will determine the priority order for entry into Tier 1 screening.

3. Tier 1 screening, a battery of *in vitro* and *in vivo* assays designed to identify those chemicals that are not likely to interact with the estrogen, androgen, or thyroid hormone systems (EAT).

4. Tier 2 testing, a battery of assays designed to determine whether a chemical may have an effect in humans similar to that of naturally occurring hormones and to identify, characterize, and quantify those effects for EAT effects.