

Executive Summary

Table of Contents

I. Introduction	<u>1</u>
II. Endocrine Disruptor Screening and Testing Advisory Committee	<u>1</u>
A. EDSTAC Formation and Structure	<u>1</u>
B. Scope	<u>2</u>
III. Chapter Three – Conceptual Framework and Principles	<u>3</u>
A. Conceptual Framework Overview	<u>3</u>
B. Recommended Principles and Guidance	<u>6</u>
IV. Chapter Four – Priority Setting	<u>6</u>
A. Initial Sorting and Phased Approach to Screening and Testing the Universe of Chemicals	<u>6</u>
B. Information Useful for Prioritization	<u>7</u>
C. High Throughput Pre-Screening	<u>8</u>
D. Endocrine Disruptor Priority Setting Database	<u>9</u>
E. Recommended Approach to Priority Setting	<u>9</u>
1. Compartment-Based Approach to Priority Setting for T1S	<u>9</u>
2. Special Compartments of Chemicals	<u>10</u>
3. Recommended Approach to Priority Setting for T2T	<u>11</u>
V. Chapter Five – Screening and Testing	<u>11</u>
A. Tier 1 Screening	<u>11</u>
1. Recommended Screening Assays	<u>11</u>
2. Criteria for Evaluating Tier 1 Results	<u>13</u>
B. Tier 2 Testing	<u>13</u>
1. Test Selection	<u>13</u>
2. Low Dose Issues in T2T	<u>14</u>
C. Validation, Standardization, Methods Development, and Research	<u>15</u>
VI. Chapter Six – Communications and Outreach	<u>15</u>
A. Principles to Guide a Communications and Outreach Strategy	<u>15</u>
B. Basic Features of a Communications and Outreach Strategy	<u>16</u>
VI. Chapter Seven – Compilation of EDSTAC Recommendations	<u>17</u>

Introduction

I.

Concerns regarding both the presence of endocrine disruptors in food, water, or other environmental media, and the potential risk they pose to humans and wildlife have been growing in recent years. Passage, in 1996, of the Food Quality Protection Act (FQPA) and Amendments to the Safe Drinking Water Act (SDWA) reflected these concerns and required 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 other such endocrine effect as the Administrator may designate.

Specifically, EPA was required to develop a screening program by August 1998; to implement the program by August 1999; and to report to Congress on the program's progress by August 2000. In 1996, EPA formed the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), charging the Committee to provide advice on how to design a screening and testing program for endocrine disrupting chemicals. This report contains the findings of the Committee, and is organized into sections discussing the EDSTAC recommendations on: a Conceptual Framework; Priority Setting; Screening and Testing; and Communications and Outreach. The Final Report contains many references to scientific papers that are intended to provide background information and/or justification for the EDSTAC's recommendations. These references reflect the EDSTAC's understanding of the science of endocrine disruption as of the final plenary, held on June 17 and 18, 1998 in Washington, DC. The Committee recognizes the science of endocrine disruption is rapidly and continually evolving and EPA will need to incorporate the results of on-going research and recent publications when implementing the Committee's recommendations.

The EDSTAC describes an endocrine disruptor as an exogenous chemical substance or mixture that alters the structure or function(s) of the endocrine system and causes adverse effects at the level of the organism, its progeny, populations, or subpopulations of organisms, based on scientific principles, data, weight-of-evidence, and the precautionary principle.

Endocrine Disruptor Screening and Testing Advisory Committee

II.

EDSTAC Formation and Structure

A.

Chapter One provides an introduction and overview of both the EDSTAC process and the report itself. The EDSTAC was composed of individuals representing various stakeholder groups and scientific expertise. The members included scientists and other representatives from: EPA, other federal agencies, state agencies, various sectors of industry, water providers, worker protection organizations, national environmental groups, environmental justice groups, public health groups,

and research scientists. The Committee began their deliberations in October 1996 and completed their recommendations in July 1998.

The Committee organized itself into the following working groups, each with a specific assignment:

- Principles Work Group to develop the EDSTAC conceptual framework (which is contained in Chapter Three);
- Priority Setting Work Group to develop a recommended approach to setting priorities for endocrine disruptor screening and testing (which are contained in Chapter Four);
- Screening and Testing Work Group to develop recommendations on the screening assays and tests to include in, and implementation of, a screening and testing program (which are contained in Chapter Five); and
- Communications and Outreach Work Group to develop recommendations on communication issues for the screening and testing program (which are contained in Chapter Six).

The work groups were comprised of Committee members as well as other individuals who were not Committee members but were asked to participate in the EDSTAC process because of their particular expertise and perspective. These work groups met periodically to accomplish their tasks. The full Committee held nine meetings, all open to the public, in different locations around the country.

B. Scope

Chapter Two describes the scope of the EDSTAC's deliberations. In addition, the chapter contains background information on the function of the endocrine system, the issue of endocrine disruptors, and the complex statutory and chemical universe within which priority setting and screening and testing must be accomplished. The primary scope of the EDSTAC was to develop recommendations for a screening and testing program for endocrine disrupting chemicals. The EDSTAC interpreted this scope to include not only the 1996 FQPA and SDWA provisions, but also those of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the Toxic Substances Control Act (TSCA), and the Federal Food, Drug, and Cosmetic Act (FFDCA). Together these acts provide testing authorities for a variety of chemicals for both human health as well as ecological effects. As a result, the EDSTAC recommended that EPA's endocrine disruptor screening and testing program (EDSTP) should:

Address both human and ecological (wildlife) effects: The EDSTAC recommended that the EDSTP scope should include screening for adverse effects to wildlife, as well as humans, recognizing that wildlife are an inherently valuable element of ecosystems and their well-being can be an indication of the overall health of the environment in which humans live.

Examine effects to estrogen, androgen, and thyroid hormone-related processes: The Committee recommended that three primary hormone systems be included in the EDSTP – estrogen, androgen, and thyroid – because they are important hormones in both humans and wildlife with a relatively large body of available relevant data and screening assays and tests from which to select. While the EDSTAC focused on these three hormones, it is aware that the science regarding the endocrine system is rapidly developing. As more data become available on other hormones, and assays are developed to identify effects on them, it is essential these additional hormones be incorporated into the screening and testing program. For example, as part of its recommendations for a phased approach to implementation, the EDSTAC calls upon EPA to periodically evaluate and, where appropriate, incorporate new screens and tests, as well as other scientific developments, into the program. In addition, the EDSTAC recommends a performance-based approach to the use of screens and tests, as well as species selection. As improved screens, tests, and/or screens/tests utilizing more appropriate species are developed and validated, the EDSTAC strongly encourages their use.

Evaluate endocrine disrupting properties of both chemical substances and common mixtures: The universe of chemicals to be prioritized for endocrine disruptor screening and testing numbers more than 87,000 and includes those listed in the TSCA Inventory, active pesticide ingredients, and ingredients in cosmetics and food additives. In addition, EDSTAC recommends that EPA should determine the technical feasibility and, where feasible, should screen and test representative samples of mixtures from six distinct types of mixtures (i.e., combinations of two or more chemicals). The inclusion of these six types of mixtures is to determine whether they may have endocrine effects, different from those of the individual component chemicals, which can only be detected when tested as a mixture.

III. Chapter Three – Conceptual Framework and Principles

A. Conceptual Framework Overview

The conceptual framework provides the structure for the EDSTAC's recommendations for screening and testing (see ES-1). The Committee determined that a tiered approach would be most effective in utilizing reasonably available resources to detect endocrine disrupting chemicals and quantify their effects. The core elements of the approach include initial sorting, priority setting, Tier 1 Screening (T1S), and Tier 2 Testing (T2T). Chapters Four and Five describe the program aspects in more detail.

Initial Sorting: A chemical entering the framework would go through initial sorting based on existing data. An evaluation and analysis of this information would direct the chemical to one of four categories. The first would lead to the "hold box" indicating the chemical is not likely to interact with the EAT hormone systems and no further analysis is required at this time. The second category contains chemicals without sufficient data to make a determination to proceed to T2T or hazard assessment. These chemicals enter the priority setting and, from there, the T1S portions of the EDSTP. The EDSTAC anticipates most chemical substances and mixtures

entering the program will fall into this category. The third category includes chemicals with sufficient existing data to move directly to T2T (i.e., existing data meet Tier 1 requirements). The fourth category includes chemicals with sufficient existing data to move directly to hazard assessment (i.e., existing data are adequate for both Tier 1 and Tier 2 requirements). In addition, the EDSTAC has included a voluntary bypass scenario, whereby the owner of a chemical could voluntarily go to T2T without having completed the full T1S battery. This bypass option brings with it a set of requirements discussed in Chapter Five.

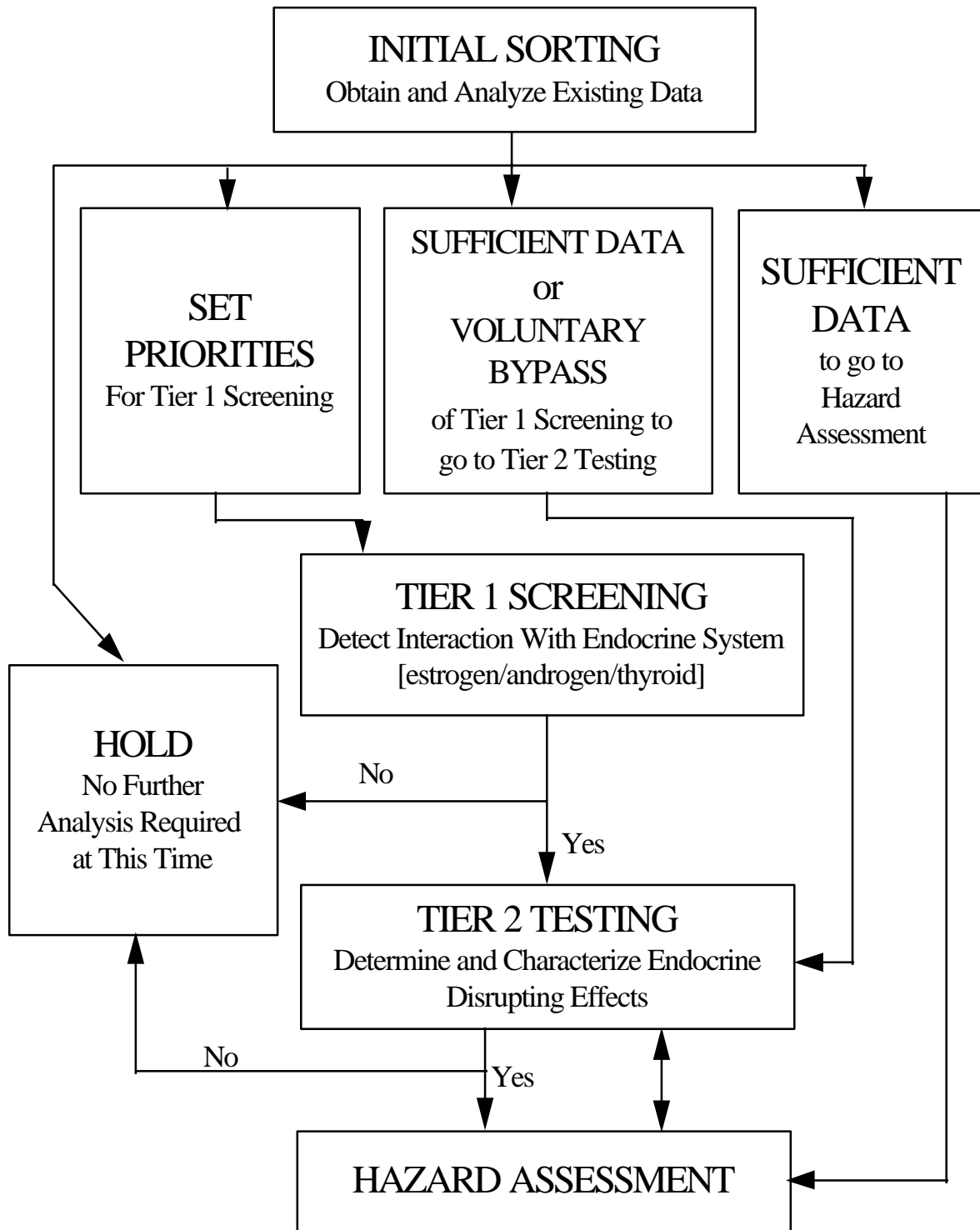
Priority Setting: The term priority setting refers primarily to the need to set priorities for the chemicals that move into T1S (the second category) after the initial sorting stage. Chemicals will be prioritized based on exposure-related information, effects-related information, and statutory criteria, and then phased into the program. This phasing of chemicals is recommended for practical reasons—the available laboratories and resources for screening and testing cannot handle the large number of chemicals entering this category simultaneously.

Tier 1 Screening: T1S is designed to detect chemical substances and mixtures capable of interacting with the EAT hormonal systems. Completion of this tier will result in either a decision to move the chemical into T2T and serve to guide test selection and dosages, or an indication that no further analysis is needed. In the latter case, the chemical would not be subjected to any further screening or testing, at that time, and would proceed to the “hold box.”

Tier 2 Testing: T2T is intended to determine whether a chemical substance or mixture exhibits endocrine-mediated adverse effects and to identify, characterize, and quantify those effects for EAT hormones. As with Tier 1, there are two possible outcomes. If endocrine mediated adverse effects are not observed, the chemical would move to the “hold box.” If such effects are observed, the information collected during T2T would be used in the Hazard Assessment process. As results are obtained, additional data may be required which would require additional testing, especially to determine whether the identified effects are endocrine-mediated.

“Hold Box:” The EDSTAC uses this term throughout the Report to mean that either no, or no further, endocrine disruptor screening and testing is necessary for a chemical substance or mixture at the time the decision is made to place the compound in the “hold box.” A chemical substance or mixture can be placed in the “hold box” at a variety of points within the recommended tiered approach to the EDSTP. These include priority setting, T1S, or T2T. As part of the EDSTAC’s recommendation for a phased approach to implementation, the EDSTAC sets forth criteria to be used during periodic evaluations of chemical substances and mixtures placed in the “hold box” to determine whether new or additional screening and testing may be necessary.

Figure ES.1. EDSTAC Conceptual Framework Providing the Structure for Screening and Testing for Endocrine Disruptors



* For a more detailed version of the initial sorting and priority setting components of this framework, please see Figure 4.1.

Recommended Principles and Guidance

B.

To guide development of the screening and testing program, the EDSTAC provided several sets of principles for general development of the conceptual framework, including:

Provisions to Bypass Tiers: The ordered sequence described above should not exclude the possibility that a chemical substance or mixture could bypass one or more tiers when information warrants such a move. In addition, the EDSTAC recommends that a voluntary bypass of T1S should be incorporated into the program; however, specific information requirements are recommended for those chemicals.

Proactive Effort to Generate Adequate Information: In cases where existing information is inadequate to determine whether a chemical substance or mixture should proceed to the next tier, there should be an active process for generating the needed information. It is anticipated that the process would be specifically tailored to the chemical under review.

Moving Through the Program: Criteria and assumptions for deciding whether a chemical should move from one tier to the next should be developed in advance of the initiation of screening and testing.

The Committee also provided additional principles to:

- guide overall development of the screening and testing strategy;
- more specifically guide decisions regarding the selection of screens and tests;
- guide how the T1S battery should be designed and used; and,
- guide how the T2T battery should be designed and used.

Chapter Four – Priority Setting

IV.

Initial Sorting and Phased Approach to Screening and Testing the Universe of

A.

Chemicals

The EDSTAC estimates the initial universe of chemicals that needs to be considered for prioritization for endocrine disruptor screening and testing numbers approximately 87,000 including: pesticides, commodity chemicals, naturally occurring non-steroidal estrogens, food additives, cosmetics, nutritional supplements, and representative mixtures. Simultaneous screening, testing, and evaluation of this universe is far beyond the capabilities of available facilities and resources. Consequently, the EDSTAC recommends both an initial sorting of this universe and a phased approach to handle the chemicals. This approach would identify high priority chemicals and permit them to proceed through the program first, followed by medium

priority chemicals, and then low priority ones. The phased approach also provides guidance to permit the bypassing of portions of the screening and testing process speeding review and evaluation.

Building on the Conceptual Framework, the EDSTAC recommends the universe of chemicals should undergo an initial sorting into the following four categories (see Figure 4.1):

1. Chemicals (primarily polymers, as described below) that are unlikely to have endocrine disrupting effects that enter the “hold box;”
2. Chemicals with insufficient data that will undergo HTPS and T1S and, possibly, T2T;
3. Chemicals with sufficient data to bypass T1S and go directly to T2T; and
4. Chemicals with sufficient data to go directly to hazard assessment.

The EDSTAC concluded that, in most cases, polymers with a number average molecular weight (NAMW) greater than 1,000 daltons are unlikely to be able to cross biological membranes and barriers and would, therefore, not be biologically available to cause endocrine-mediated effects. Consequently, the Committee recommends exempting the approximately 25,000 polymers that meet this NAMW criterion from screening and testing by placing them in the “hold box,” pending review of their components. The EDSTAC further estimates that the number of chemical substances that fall into categories 3 and 4 probably number no more than 1,000.

Thus, the EDSTAC estimates that approximately 62,000 chemicals will remain and need to be at least considered for screening and, if necessary, testing. The EDSTAC recognizes, however, that it is not likely to be possible or necessary to screen and test 62,000 chemicals. Although the EDSTAC incorporated a phased approach to implementation in order to address the volume of chemicals ultimately needing to be screened and tested, the EDSTAC did not define the number of phases, how long each phase should be, or the number of chemicals that should be screened and/or tested in each phase.

Information Useful for Prioritization

B.

The EDSTAC recommends that the following types of exposure- and effects-related information be used to prioritize chemicals for T1S. Exposure-related information includes:

- Biological sampling data (e.g., blood or tissue analyses) for humans and other biota;
- Environmental, occupational, consumer product, and food-related data;
- Data on environmental releases;
- Production volume; and
- Fate and transport data and models.

Sources of data on effects-related information are:

- Toxicological laboratory studies and databases;
- Epidemiological and field studies and databases;
- Predictive biological activity or effects models (e.g., SARs, QSARs); and
- Results of high throughput pre-screening.

The EDSTAC presents a detailed discussion of the strengths and limitations, as well as guiding principles, for using each of these information categories for the purpose of priority setting.

C. High Throughput Pre-Screening

The EDSTAC recognized that biological effects data are incomplete or lacking for most chemicals, a condition which makes priority setting difficult. To help address this problem, the EDSTAC recommends that some of the T1S assays be conducted in a high-speed, automated fashion to provide preliminary hormonal or biological activity information. This approach is called “high throughput pre-screening” (HTPS) where, rather than following traditional manual sample preparation, handling, and analysis procedures, automated techniques and robotics are used to accelerate the assay process. Such a process permits a large volume of chemicals to be tested in a short period of time. The EDSTAC recommends that HTPS be conducted on: (1) all chemicals with current production volumes greater than 10,000 pounds per year (estimated to be approximately 15,000 chemicals); (2) all pesticide active ingredients and formulation inerts ; and (3) all chemicals that are proposed to bypass either T1S or both T1S and T2T for any reason.

The EDSTAC recommends that the T1S *in vitro* transcriptional activation assays be modified and validated for use in the high throughput mode. It further recommends that, when used in the screening and testing program, the HTPS assays should:

- provide information about the ability of chemicals to bind to the estrogen, androgen, or thyroid hormone receptors;
- be used with other exposure- and effects-related data in prioritizing chemicals for T1S;
- improve QSAR predictive models; and
- provide information to assist in the design of the tests in T2T for chemicals bypassing T1S.

The EDSTAC has recommended, and EPA has already initiated, a feasibility demonstration pilot program be created to assess the proposed use of HTPS. The EDSTAC’s recommendations are, therefore, contingent upon technical feasibility of the HTPS technology and successful standardization and validation of the HTPS assays. If HTPS is technically feasible and validation is successful, the EDSTAC believes that HTPS can be a powerful, cost effective tool in the EDSTP.

Endocrine Disruptor Priority Setting Database

D.

The EDSTAC recommends that data used as the basis for sorting and priority setting be organized into a relational database called the Endocrine Disruptor Priority Setting Database (EDPSD). Development of this database was initiated by the EDSTAC but, due to time and resource limitations, was not completed. The EDSTAC recommends that EPA complete and maintain the EDPSD. The EDPSD can be an invaluable resource for initial sorting and priority setting provided it contains current data and its use is simple and open to review by all.

The EDSTAC recommends that the EDPSD development proceed in three stages:

1. capture data from existing databases such as use data on fate, transport, and toxicity;
2. use data not readily available in existing databases such as chemicals and concentrations from the National Health and Nutrition Examination Survey and the Hazardous Substances Emergency Events Surveillance; and
3. incorporate HTPS data and improved Quantitative Structure Activity Relationships (QSAR) models.

Recommended Approach to Priority Setting

E.

Compartment-Based Approach to Priority Setting for T1S

1.

In general, the EDSTAC recommends that whatever priority setting approach is used by EPA it should be open and simple. The EDSTAC further recommends that, while the process should be driven by empirical data, it should allow for chemicals of concern, which have less data, especially less effects-related data, to be included in the higher priority rankings.

Based on these principles, the EDSTAC recommends that EPA use a “compartment-based” approach to priority setting that builds directly upon the recommended exposure- and effects-related information categories described above. The term “compartment-based” refers to an approach whereby different combinations of information, and criteria that flow from this information, are used to generate a set of priorities for each phase of the program. The EDSTAC recommends four broad categories of compartments should be developed, including those that: (1) would be based on the integration of exposure and effects information and criteria; (2) rely only on exposure-related information and criteria; (3) rely only on effects-related information and criteria; and (4) focus on special compartments of chemicals.

While the EDSTAC has not agreed upon specific compartments, nor the order in which they should be used in priority setting, it did provide a number of illustrations to show how the compartment-based approach might be used in practice. The EDSTAC also made the following recommendations for the development of the compartment-based approach:

- have a multi-stakeholder group use the EDPSD to characterize and define what will be included in each compartment;
 - determine whether and, if so, how the compartments should be weighted; and
 - address the possibility of overlaps between compartments.
2. Special Compartments of Chemicals

The EDSTAC identified a number of specially targeted compartments for purposes of priority setting which include:

Nominations: The priority setting process recommended by the EDSTAC 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. The EDSTAC recommends EPA establish a parallel but separate priority setting process where chemicals with regional or local exposure can be nominated by affected citizens to receive a priority for T1S. The EDSTAC recommends that a goal for each phase of the EDSTP is for no less than 5% of the total number of chemicals be drawn from those that are nominated but not otherwise selected in the core process as a high priority for T1S. The EDSTAC recognizes that the total number of nominations or their quality may be such that this goal cannot be met in specific phases. The EDSTAC further recommends the use of a specialized set of criteria for prioritizing nominated chemicals that would focus primarily on exposure and, secondarily, on available effects data. The EDSTAC also sets forth recommendations on the procedures EPA should use for submitting a nomination and, where necessary, protecting the identity of the person submitting the nominations.

Mixtures: The EDSTAC felt that mixtures, defined as a combination of two or more chemicals, needed special attention during the initial stages of sorting and prioritization. Consequently, the EDSTAC recommends that EPA should determine the technical feasibility and, where feasible, should 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.

Some of the technical challenges for screening and testing mixtures mirror those of single compounds, however, the EDSTAC acknowledges that the technical feasibility of screening and testing mixtures is by no means certain. Technical feasibility for screening and testing mixtures 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, T1S, and T2T depending on their physical properties, and validation and standardization of the results.

Naturally Occurring Non-Steroidal Estrogens (NONEs): Another special class of chemicals of concern to the EDSTAC are naturally occurring non-steroidal estrogens. 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. EDSTAC recommends that EPA include representatives from the seven chemical classes of NONEs in the EDSTP.

3. Recommended Approach to Priority Setting for T2T

While the EDSTAC recommended a compartment-based approach to setting priorities for T1S, it also recommended that priority setting for T2T for chemicals that bypass T1S should be done as follows:

- Food use pesticides should use the schedule EPA has established for tolerance reassessments and pesticide re-registration under the FQPA.
- For all other chemicals voluntarily bypassing T1S, priorities should be established on a case-specific basis using all available information, including the priority ranking for T1S. The EDSTAC recommends that priority setting for these chemicals should be generally driven by the same priorities as those set during the priority setting phase of the EDSTP, and that voluntary action on the part of owners/producers should expedite, but not delay, testing.

The EDSTAC did not develop an explicit set of recommendations for how to set priorities for chemicals that produce positive results in T1S and must move forward into T2T.

V. Chapter Five – Screening and Testing

A. Tier 1 Screening

1. Recommended Screening Assays

The screening tier of the Conceptual Framework detects whether a chemical substance or mixture may interact with the endocrine system for estrogen, androgen, and thyroid hormones. The EDSTAC developed several criteria to guide the selection of T1S assays:

- maximize sensitivity which serves to minimize false negatives;
- include a range of organisms representing differences in metabolism;
- detect all known modes of action for the endocrine endpoints of concern;
- include a sufficient range of taxonomic groups among the test organisms; and

- incorporate sufficient diversity among the endpoints, permitting weight-of-evidence conclusions.

The EDSTAC recommends both *in vitro* and *in vivo* assays be included in T1S. Each type of assay has strengths and weaknesses but when used in combination, the weaknesses can be minimized. Those chemical substances and mixtures which go through the HTPS program, if it is technically feasible and validated, would not be required to do the first two *in vitro* assays at the bench. The recommended assays include:

- *In Vitro* Assays
 - Estrogen Receptor Binding/Reporter Gene Assay
 - Androgen Receptor Binding/Reporter Gene Assay
 - Steroidogenesis Assay with minced testis
- *In Vivo* Assays
 - Rodent 3-day Uterotrophic Assay
 - Rodent 20-day Pubertal Female with thyroid
 - Rodent 5-7-day Hershberger Assay
 - Frog Metamorphosis Assay
 - Fish Gonadal Recrudescence Assay

The battery of T1S assays is designed to work as a whole. The EDSTAC believes that data from all the assays are necessary if EPA is to make accurate decisions about the chemicals that are screened. The Committee also believes this battery of assays meet all the established criteria for T1S but the assays must be validated and standardized before final inclusion in the T1S battery. At present no T1S assay is fully validated.

The EDSTAC believes the recommended T1S battery, if validated, will have the necessary breadth and depth to detect any currently known endocrine disruptors. The EDSTAC recognizes that chemical substances or mixtures might produce effects from prenatal/pre-hatch exposure that would not be detected from pubertal or adult exposures. To address these concerns, the EDSTAC recommends that EPA take affirmative steps, in collaboration with industry and other interested parties to attempt to develop the protocol for a full life cycle (i.e., with embryonic exposure and evaluation of the adult offspring) developmental exposure screening assay that can be subjected to validation and standardization.

The EDSTAC also recommends four assays be examined as possible alternatives to some of those proposed above:

- *In Vitro*
 - Placental Aromatase Assay

- *In Vivo*
 - Modified Rodent 3-day Uterotrophic Assay (intraperitoneal dosing);
 - Rodent 14-day Intact Adult Male Assay with thyroid; and
 - Rodent 20-day Thyroid/Pubertal Male Assay.

These alternatives should also be included in the validation and standardization program. If they are at least as sensitive as the assays proposed for the T1S battery, they might replace some of the recommended assays, particularly if they offer reductions in time, cost, and complexity of T1S.

2. Criteria for Evaluating Tier 1 Results

The EDSTAC recommends that a weight-of-evidence approach be used to evaluate T1S results and make decisions about going on to T2T. This approach would include: (1) the balance of positive and negative responses observed in both the *in vitro* and *in vivo* assays; (2) the nature and range of the biological effects observed; (3) the shape of the dose-response curves; (4) the severity and magnitude of effects induced; and (5) the presence or absence of response in multiple taxa. Ten principles were recommended for evaluating the T1S results under a weight-of-evidence approach.

The evaluation of T1S data, and other available information (e.g., HTPS or literature data), will result in a decision either that the chemical needs no further STET and can move to the “hold box” or that the chemical needs to be tested in Tier 2.

B. Tier 2 Testing

1. Test Selection

The purpose of T2T is to determine whether a chemical substance or mixture exhibits endocrine-mediated adverse effects and to identify, characterize, and quantify those effects for EAT hormones. The EDSTAC identified three principles to guide selection of tests for inclusion in T2T:

- Tests must include the most sensitive developmental lifestage.
- Tests must identify the specific hazard caused by the chemical and establish a dose-response relationship.
- A range of taxa must be included in Tier 2 tests.

With these criteria in mind, the EDSTAC recommends that the following tests be incorporated into Tier 2:

- Two-Generation Mammalian Reproductive Toxicity Study or a less comprehensive test (e.g., Alternative Mammalian Reproductive Test)
- Avian Reproduction Test
- Fish Life Cycle Test
- Mysid Life Cycle Test
- Amphibian Development and Reproduction Test

As with T1S, the battery of Tier 2 tests is designed to work as a whole. The outcome of T2T is designed to be conclusive in relation to the outcome of T1S, and any other prior information, in the sense that a negative outcome in T2T will supersede a positive outcome in T1S. Furthermore, each full test in T2T has been designed to include those endpoints that will allow one to reach 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 T2T battery would provide a more comprehensive profile of the effects a chemical substance or mixture could induce via EAT endocrine disruption mode(s)/mechanism(s) of action than would be the case if only a subset of tests were performed. The EDSTAC recommends that the “default” action, in the absence of any prior information, should be to perform all tests in the T2T battery with all endpoints.

However, performance of the entire battery with multiple generations may not always be necessary. Therefore, the EDSTAC developed guidance in the selection of Tier 2 tests, focusing upon: (1) the determination of which of the five taxonomic groups should be included in the Tier 2 testing of a specific chemical substance or mixture; (2) the circumstances under which it may be appropriate to perform an alternative test, with a particular focus on the selection of alternative mammalian tests; (3) the selection of endpoints; (4) the special case of chemicals that bypass T1S and go directly to T2T; and (5) the potential need for supplemental information to complete T2T.

The Committee believes that while this battery of assays meets all the established T2T criteria and objectives, the test protocols must be validated and standardized before final incorporation into the screening and testing program. At present, none of the new tests or enhancements to existing test guidelines are fully validated or standardized.

2. Low Dose Issues in T2T

The EDSTAC recognized that questions have been raised as to the adequacy of conventional toxicology study designs for assessment of endocrine active substances, particularly with regard to low dose selection and the identification of no-observed-adverse-effect-levels (NOAEL). To address these questions, the EDSTAC recommends that a project be performed to resolve the underlying uncertainties and controversy about these issues. The purpose of the project is to address the nature of the dose-response curves for exogenous estrogenic substances in order to allow more informed judgments about appropriate toxicology study designs for substances that have hormonal activity. In addition, the EDSTAC summarized their preliminary discussions

regarding the exact design of the research program and laid out implications and corresponding actions to be taken based upon the possible results of the research.

C. Validation, Standardization, Methods Development, and Research

The EDSTAC believes validation and standardization of the recommended screens and tests are essential for implementation of the EDSTP. EDSTAC also believes the validation and standardization program is of highest priority, and recommends that it proceed on an accelerated schedule. The EDSTAC recommends that the validation and standardization program be consistent with the principles articulated by the national (ICCVAM, 1996; Zeiger, 1998) and international (OECD, 1996) alternative methods validation groups. As mentioned throughout Chapter Five, each assay and test recommended for T1S or T2T needs some level of validation, standardization, methods development, or further research before being accepted as a regulatory toxicity screen or test for inclusion in the EDSTP. The level of effort needed to fully validate and standardize may be different for each individual assay or test (including all recommended endpoints) for each individual assay or test. The effort required for each assay or test will be defined by a variety of criteria including: period of time in use, existing level of general acceptance in the endocrine toxicology field, and existing understanding of relevancy and reliability. Regardless of the effort required, the EDSTAC believes all of the assays and tests recommended for T1S and T2T must be fully validated and standardized before being included in the EDSTP. The EDSTAC recommends that as individual assays and tests are validated and standardized, they can be utilized in the EDSTP without waiting for all assays and tests in the batteries to be validated. EDSTAC further recommends that a multi-stakeholder process, involving government, industry, and academia, be utilized in validating and standardizing the T1S and T2T batteries.

VI. Chapter Six – Communications and Outreach

A. Principles to Guide a Communications and Outreach Strategy

Good communication is essential for the success of the screening and testing program. Particular care is needed to ensure that, to the extent possible, potential misuse of information generated by the EDSTP does not occur. EPA should develop a strategy for clear and accurate communication to all the stakeholders during the development and implementation of the screening and testing program. It is of particular importance that EPA clearly communicate the limitations that must be placed on the results of the screening and testing as well as the meaning and implications of the decisions made by the Agency based upon these results.

The EDSTAC recommends that five principles be used in developing a communication and outreach strategy for the screening and testing program:

- the process and results of the program should be clear and open;
- the results should be communicated within the context set forth in the EDSTAC final report;
- the limitations and uncertainties associated with the available data and the screening and testing results should be clearly articulated;
- any changes in the program should be promptly and clearly communicated; and
- a quality assurance program should be developed to ensure that the Endocrine Disruptor Priority Setting Database is current and accurate.

Basic Features of a Communications and Outreach Strategy

B.

The EPA strategy should address: (1) what should be communicated; (2) to whom it should be communicated; (3) how it should be communicated; and (4) when it should be communicated. Specifically, the EDSTAC recommends that EPA communicate information on the screening and testing approach, the status and results for chemicals that have been evaluated in the program, and the nominations process. This information should be communicated to all stakeholders who have expressed an interest in the program, including the general public. To facilitate communications, it is recommended that EPA tailor the information to specific target audiences. Some examples of target audiences include, but are not limited to, farm workers, environmental justice organizations, or industries that formulate products but do not manufacture the component chemicals, often referred to as “downstream” industries.

The Committee recommends information be communicated in a variety of ways. EPA should develop a tracking system for chemicals entering the EDSTP that is compatible and fully integrated with the Endocrine Disruptor Priority Setting Database described in Chapter Four. This database should not exist in isolation; rather it should be integrated into those being developed elsewhere in the Agency. The tracking system should be designed to enable the public to have quick access to determine the status of a chemical. Access to information about the EDSTP should be available via Internet, telephone, fax, mail, and the Federal Register.

Finally, information should be made available on a regular basis using a bulletin or newsletter of limited length. This update could summarize the status of the overall program and individual chemicals that have entered it as well as important developments or changes in the program. It could also be a vehicle where EPA could issue a call for nominations. EPA will need to commit adequate resources to implement a newsletter and the other EDSTAC communications and outreach recommendations.

VI. Chapter Seven – Compilation of EDSTAC Recommendations

Chapter Seven includes all of the Committee's recommendations made in Chapters Three, Four, Five, and Six. Each set of recommendations can also be found at the end of their respective chapters.