2.0 PROPOSED SUBSTANCES[1] AND SELECTION CRITERIA FOR VALIDATION OF *IN VITRO* ENDOCRINE DISRUPTOR SCREENING ASSAYS

2.1 Introduction

To facilitate the validation of *in vitro* ER and AR binding and TA assays, ICCVAM has compiled a list of 78 substances recommended for use in future validation studies. Versions of this list specific to each type of assay are provided in **Sections 3.0** through **6.0**. Each version includes the available quantitative and qualitative data for each substance, and its known or anticipated qualitative response in the assay type being considered. The available data are based on information compiled in the four BRDs, as well as information found in publications reviewed or published after completion of the BRDs. A number of factors and criteria were considered in compiling this list, including the recommendations of the four draft BRDs, the Expert Panel, and the EDWG, as well as substances proposed for *in vitro* endocrine disruptor testing by the EPA. To allow for a direct comparison between results obtained from *in vitro* and *in vivo* endocrine disruptor test methods, the list also includes substances proposed for *in vivo* endocrine disruptor testing by EPA and OECD.

2.2 Draft Background Review Document Recommendations

Each of the four draft BRDs included a list of substances recommended for future validation studies of the assay type considered. The number of substances included in each list are provided in **Table 2–1**. Selection of these substances was based on:

- the availability of published or submitted data demonstrating reproducible positive or negative responses in multiple studies and/or test methods;
- the extent to which these substances covered the range of negative to weakly positive to strongly positive responses; and
- the distribution of the proposed substances among chemical classes.

2.3 Expert Panel Recommendations on Proposed Substances for Validation Studies[2]

As described in **Section 1.1.1**, an Expert Panel developed recommendations on the adequacy and appropriateness of the substances recommended in the draft BRDs for use in future validation studies. The Expert Panel generally agreed with the lists of proposed substances but also recommended that:

- for a specific receptor (ER or AR), the same substances should be tested in binding and TA agonism and antagonism assays;
- the proportion of negative substances in each list should be increased to at least 25% of the total number of substances to better evaluate test method specificity;
- an ER binding substance with a potency two orders of magnitude lower than 17β-estradiol should be included as a concurrent

[1]Inclusion of a substance does not mean that EPA, NICEATM, ICCVAM, or the Expert Panel has or will make a determination that any use of the substance will pose a significant risk. Further, these substances should not be interpreted to be "endocrine disruptors"; the substances listed are simply compounds that have been, or may prove to be useful in developing, standardizing, or validating screening and testing methods.

[2]Expert Panel Evaluation of the Validation Status of *In Vitro* Test Methods for Detecting Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays. Expert Panel Final Report, September 2002. Report available in **Appendix A** of this document.

positive control in *in vitro* ER binding assays;
- substances (e.g., actinomycin D, cycloheximide, sodium azide, 12-*O*-tetradecanoylphorbol-13-acetate) that might interfere indirectly with reporter gene transcriptional activation by altering metabolic pathways, such as RNA and protein synthesis, should be included;
- additional substances from underrepresented chemical classes (e.g., phthalates, polycyclic aromatic hydrocarbons [PAHs], polychlorinated biphenyls) should be included; and
- a central repository should be organized to provide substances of high purity for use in future validation studies.

2.4 ICCVAM, EDWG, and NICEATM Proposed List of Substances for Validation

The EDWG subsequently reviewed the Expert Panel's recommendations regarding substances that should be used in future validation studies and, in collaboration with NICEATM, developed a revised list of proposed substances. A challenging task was meeting the recommendation of the Expert Panel that at least 25% of the substances proposed for validation studies be negative for binding or TA for the receptor being used. During the preparation of the BRDs, only a few substances had been identified as consistently negative for the endpoint of interest in multiple studies (**Table 2-1**). However, on the assumption that some of the substances positive in ER binding or TA assays would likely be negative in the corresponding AR-based assays (and vice versa), it was decided that such substances could serve as presumptive negatives in the alternative receptor-based assays. This approach would also minimize the total number of different chemicals to be included in an endocrine disruptor chemical repository.

2.4.1 Candidate Substances

Initially, 122 candidate substances were identified for validation studies; this list was subsequently reduced to 78 substances. The 122 candidate substances consisted of:
- the 85 substances recommended in the four BRDs for future validation studies (see **Section 12.0**, **Table 12-1** in the ER and AR Binding Assay BRDs, and **Section 12.0**, **Tables 12–1** and **12–2** in the ER and AR TA BRDs) (NIEHS 2002a, 2002b, 2002c, 2002d);
- the 44 substances scheduled for testing in *in vivo* mammalian endocrine disruptor assays by the EPA and the OECD[3], 22 of which had been included in the lists provided in the BRDs. The *in vivo* list included five substances (oxazepam, phenobarbital, L-thyroxine, ammonium perchlorate, and propylthiouracil) that are known to disrupt thyroid function *in vivo* and thus could likely serve as presumed negative substances in *in vitro* ER and AR binding and TA assay validation studies;
- the 38 substances scheduled for testing in *in vitro* endocrine disruptor assays by the EPA, 29 of which had been included in the lists provided in the BRDs; and
- the 6 additional substances recommended by the Expert Panel.

Five of the candidate substances (butylbenzyl phthalate, diethylhexyl phthalate, dibenzo[*a,h*]anthracene, fluoranthene, and zearalenone) belong to chemical classes that had been underrepresented in the BRD

[3]On July 8, 2002, NICEATM received a list of the substances selected or recommended for *in vitro* endocrine disruptor testing by the EPA and for *in vitro* and *in vivo* endocrine disruptor testing by the EPA or the OECD from Mr. Gary E. Timm in the EPA Office of Science Coordination and Policy, Washington, DC. The list was compiled by Mr. James Kariya for presentation at the March 2002 meeting of the EPA EDMVS.

lists (phthalates for the first two substances, PAHs for the second two substances, and resorcylic acid lactone/phenol for the last substance). In addition, seven of the candidate substances (bisphenol A, 1,1-dichloro-*bis*[4-chlorophenyl]ethylene, dichlorodiphenyltrichloroethane, di-(2-ethylhexyl)phthalate, di-*n*-butylphthalate, nonylphenol, and octylphenol) have been tested *in vivo* for endocrine disruptor activity by the Japanese Ministry of Health (JME). The JME website *http://www.env.go.jp/en/topic/edcs.html* provides details on the specific *in vivo* test methods in which these substances were tested and the results obtained.

2.4.2 Selection of 78 Proposed Substances

The list of 122 candidate substances was reduced to 114 candidates based on the following:

- methyl parathion and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, highly toxic substances proposed by EPA for *in vivo* testing, were excluded to avoid potential worker exposure;
- 4-chloro-4'-biphenylol and 2',4',6'-trichloro-4-biphenylyol, two substances recommended in the draft BRDs, and Arochlor 1254, a substance proposed for *in vivo* testing by the EPA, were excluded because of hazardous waste disposal concerns;
- letrozole was excluded because EPA was not sure that it would be tested *in vivo* and because of the absence of *in vitro* data;
- testosterone propionate, also proposed for *in vivo* testing by EPA, was excluded because it is readily hydrolyzed *in vivo* to its parent compound, testosterone, which has been tested much more extensively in multiple *in vitro* endocrine disruptor assays; and
- tamoxifen citrate, proposed by the EPA for *in vitro* testing, was excluded because its parent compound, tamoxifen, has been tested much more extensively in multiple *in vitro* endocrine disruptor assays.

The remaining list of 114 candidate substances was reduced to 78 substances by excluding substances not scheduled for *in vitro* testing by the EPA or *in vivo* testing by EPA and OECD (with the exceptions noted above). Thus, 39 of the 44 substances proposed for *in vivo* testing by EPA and OECD are included in this list, as well as 37 of the 38 substances proposed for *in vitro* testing by EPA.

The expected performance of these 78 substances in the various *in vitro* endocrine disruptor assays is provided in **Table 2-2A** for *in vitro* ER-based assays and **Table 2-2B** for *in vitro* AR-based assays. Based on the available data, about 47% and 56% of the substances are expected to be negative in *in vitro* ER- and AR-based assays, respectively. Among these 78 substances, 70 chemical and 13 product classes are represented. Not all 78 substances could be assigned to a product class. The distribution of substances among chemical and product classes is provided in **Tables 2-3** and **2-4,** respectively, while **Table 2-5** provides information on the chemical and product classes assigned to each of the recommended 78 substances.

2.4.3 Purpose and Advantages of the List of 78 Substances

The current goal of the EPA is to validate *in vitro* ER and AR binding and TA assays as components of the EDSP Tier 1 screening battery, which includes both *in vitro* and *in vivo* assays. The purpose of the list of 78 substances is to ensure that the comparative reliability and performance of *in vitro* ER and AR binding and TA assays are adequately characterized across a broad range of chemical classes and responses. Inclusion in this list of many of the substances proposed for the validation of Tier 1 and Tier 2 *in vivo*

assays will help characterize the usefulness of the Tier 1 screening battery for prioritizing substances for Tier 2 testing, and hopefully facilitate development of more predictive *in vitro* endocrine disruptor assays. The current proportion of negative and presumed negative substances in this list is greater than the 25% recommended by the Expert Panel. However, for most of the negative substances, the classification of negative is not based on actual data, and, despite expectations to the contrary, a number of substances expected to be discordant for activity between ER- and AR-based assays have been reported as active in both.

2.4.4 Minimum Lists of Substances for Validation of *In Vitro* Endocrine Disruptor Assays

Because the purpose of these *in vitro* assays in the Tier 1 screening battery is to provide binding and TA data that will be considered in a weight-of-evidence evaluation to prioritize substances for Tier 2 testing, characterizing the activity of all of the substances expected to be negative *in vitro* (e.g., thyroid disruptors, aromatase inhibitors) may not be essential. Thus, ICCVAM developed minimum lists of substances that should be given priority during the validation of *in vitro* ER and AR binding and TA assays. For each receptor type, the same substances are proposed for testing in binding and TA (agonist and antagonist) assays. This approach will allow for a direct comparison of the reliability and performance of these different types of *in vitro* endocrine disruptor assays. The substances proposed in the BRDs and those being tested by the EPA in *in vitro* assays have been used as the foundation for each minimum list. Additional substances recommended by the Expert Panel (see **Section 2.3**), and those likely to be negative for the endpoint being assessed, complete the lists.

The minimum lists contain 53 substances[4] for ER binding and TA assays and 44 substances[5] for AR binding and TA assays, with similar distributions of substances across the ranges of responsiveness and chemical classes as contained in the list of 78 substances. For ER binding, ER TA agonism, and ER TA antagonism assays, 40 (75%), 34 (64%), and 11 (21%) substances, respectively, are positive or presumed positive, and 13 (25%), 19 (36%), and 42 (79%), respectively, are negative or presumed negative in each assay. For AR binding, AR TA agonism, and AR TA antagonism assays, 33 (75%), 20 (45%) and 20 (45%) substances, respectively, are positive or presumed positive, and 11 (25%), 24 (55%), and 24 (55%), respectively, are presumed negative in each assay. These 53 and 44 substances selected for the minimum lists are in bold type in the appropriate tables in **Sections 3.2, 4.2, 5.2,** and **6.2**.

2.4.5 Data Supporting the Recommended Substances

The data provided with the substance lists in **Sections 3.0** through **6.0** summarize information obtained primarily from peer-reviewed scientific reports and, secondarily, from two reports of unpublished *in vitro* TA test method data. These latter reports were received from Otsuka Pharmaceutical Co., Ltd. (Tokushima, Japan), and from Xenobiotic Detection Systems, Inc. (Durham, North Carolina). Of the 78 substances included in the primary list, relevant quantitative data from *in vitro* ER and AR binding studies are available for 45 (58%) and 33 (42%) of the substances, respectively. For *in vitro* ER TA assays, relevant quantitative or qualitative data from agonist and antagonist studies are

[4] This substance total excludes the reference estrogen, 17β-estradiol.

[5] This substance total excludes the reference androgen, methyltrienolone.

available for 45 (58%) and 18 (23 %) of the substances, respectively. For *in vitro* AR TA assays, relevant quantitative or qualitative data from agonist and antagonist studies are available for 45 (58%) and 27 (35%) of the substances, respectively. Many of these substances were tested in only one or two of the four types of assays and often once only. Thus, there are numerous data gaps, as well as incomplete information, regarding how the different types of *in vitro* ER- and AR-based assays will respond to the 78 recommended substances.

Because the data were generated by studies conducted by different laboratories using different experimental protocols, the data are highly variable and, thus, should not be used as definitive target values to be obtained during future validation studies. The intent of the data summaries presented in **Sections 3.2, 4.2, 5.2,** and **6.2** is to inform interested investigators of the published quantitative and qualitative responses obtained for these substances in the four types of assays. Moreover, although the anticipated responses assigned to substances lacking data are supported by indirect evidence in the literature, these assigned responses may prove to be inaccurate.

Table 2–1: Numbers of Substances Recommended in the BRDs for the Validation of *In Vitro* ER and AR Binding and TA Assays

In Vitro Assay Type	Number of Substances	Number of Positive Substances	Number of Negative Substances
ER Binding	33	30 (91%)	3 (9%)
ER TA Agonism	31	25 (81%)	6 (19%)
ER TA Antagonism	20	16 (80%)	4 (20%)
AR Binding	31	28 (90%)	3 (10%)
AR TA Agonism	28	18 (64%)	10 (36%)
AR TA Antagonism	24	20 (83%)	4 (17%)

Table 2–2: Distribution of Anticipated Responses of the 78 Recommended Test Substances in *In Vitro* ER and AR Binding and TA Assays

A. *In Vitro* ER-Based Assays [a]

Expected Response	ER Binding	ER TA	
		Agonism	Antagonism
Positive[b] and Presumed Positive[c]	41 (53%)	35 (45%)	11 (14%)
Negative[d] and Presumed Negative[e]	37 (47%)	43 (55%)	67 (86%)
Total	78	78	78

[a] Based on information provided in **Sections 3.0** through **6.0**. Counts include the recommended reference estrogen, 17β-estradiol.

[b] Substances that tested positive for ER binding or ER TA in >50% of multiple studies conducted.

[c] Substances that tested positive in ≤50% of reported ER binding or ER TA studies; that tested positive in the only study conducted; or that have no relevant receptor binding or TA data available for the test method of interest but which are presumed positive based on their known mechanism of action or their responses in other endocrine disruptor screening assays (e.g., methyl testosterone, an ER agonist, is presumed positive in ER binding assays).

[d] Substances that tested negative for ER binding or ER TA in multiple studies, when tested up to the limit dose.

[e] Substances that tested negative but had not been tested in multiple ER binding or in multiple ER TA studies up to the limit dose (i.e., 1 mM); or that have no relevant receptor binding or TA data available for the test method of interest but which are presumed negative based on their known mechanism of action or their responses in other endocrine disruptor screening assays (e.g., anastrazole and fadrozole, known aromatase inhibitors, are presumed negative in ER binding and TA assays).

Table 2–2: Distribution of Anticipated Responses of the 78 Recommended Test Substances in *In Vitro* ER and AR Binding and TA Assays *(continued)*

B. *In Vitro* AR-Based Assays [a]

Expected Response	AR Binding	AR TA	
		Agonism	Antagonism
Positive[b] and Presumed Positive[c]	34 (44%)	22 (28%)	21 (27%)
Negative[d]	44 (56%)	56 (72%)	57 (73%)
Total	78	78	78

[a] Based on information provided in **Sections 3.0** through **6.0**. Counts include the recommended reference androgen, methyltrienolone.

[b] Substances that tested positive for AR binding or AR TA in >50% of multiple studies conducted.

[c] Substances that tested positive in ≤50% of reported AR binding or AR TA studies; that tested positive in the only study conducted; or that have no relevant receptor binding or TA data available for the test method of interest but which are presumed positive based on their known mechanism of action or their responses in other endocrine disruptor screening assays (e.g., ketoconazole, an AR agonist, is presumed positive in AR binding assays).

[d] Substances that tested negative but had not been tested in multiple AR binding or in multiple AR TA studies up to the limit dose (i.e., 1 mM); or that have no relevant receptor binding or TA data available for the test method of interest but which are presumed negative based on their known mechanism of action or their responses in other endocrine disruptor screening assays (e.g., anastrazole and fadrozole, known aromatase inhibitors, are presumed negative in AR binding and TA assays). No substances could be classified as negative for AR binding or AR TA since none had been tested in multiple studies at or above the limit dose of 1 mM recommended in **Sections 5.1.5** and **6.1.3**.

Table 2–3: Distribution of the 78 Recommended Substances Among Chemical Classes [a]

Chemical Class [b]	Number of Substances Selected for Validation Studies [c]	Chemical Class [b]	Number of Substances Selected for Validation Studies [c]
Alkylphenol	2	Estrene	6
Amide	2	Flavanoid	6
Androstene	2	Flavone	4
Anilide	3	Fluorene	1
Anthracene	1	Glutaramide	1
Aromatic amine	1	Heterocycle	5
Aromatic amino acid	1	Imidazole	4
Arylamine	1	Isoflavone	2
Azide	1	Ketone	2
Benzimidazole	1	Lactone	1
Benzodiazepine	1	Nitrile	5
Benzopyranone	1	Nitrobenzene	2
Benzylidene	3	Norpregnene	1
Bisphenol	3	Organic acid	2
Butyrophenone	1	Organic salt	2
Carbamate	1	Organochlorine	8
Chlorinated aromatic hydrocarbon	1	Paraben	1
Chlorinated bridged cycloalkane	1	Peptide	1
Chlorinated hydrocarbon	1	Phenol	14
Chlorinated triphenylethylene	1	Phenoxazone	1
Coumarin	1	Phorbol ester	1
Coumestan	1	Phthalate	3
Cyclic imide	2	Piperazine	2
Diphenylalkane	3	Piperidine	2
Diphenylalkene	3	Polycyclic aromatic hydrocarbon	2
Diphenyalkanecarboxylic acid	1	Polycyclic hydrocarbon	1
Diphenyl ether	1	Pregnenedione	1

Table 2–3: Distribution of the 78 Recommended Substances Among Chemical Classes [a]
(continued)

Chemical Class [b]	Number of Substances Selected for Validation Studies [c]	Chemical Class [b]	Number of Substances Selected for Validation Studies [c]
Pregnene lactone	1	Terpene	1
Pyrimidine	3	Triazine	1
Quinoline	1	Triazole	1
Resorcylic acid lactone	1	Triphenylethylene	2
Steroid, nonphenolic	15	Triphenylmethane	1
Steroid, phenolic	5	Uracil	1
Stilbene	3	Urea	1
Sulfone	1	Yohimban	1

[a] Based on information provided in **Table 2-5**.

[b] Substances were assigned to chemical classes based on available information from standardized references (e.g., *The Merck Index* and the U.S. National Library of Medicine's ChemID database) and from an assessment of chemical structure.

[c] Because a substance may be included in more than one chemical class, the number of substances selected for validation studies totaled across chemical classes exceeds the number of selected substances.

Table 2–4: Distribution of the 78 Recommended Substances Among Product Classes [a]

Product Class [b]	Number of Substances Selected for Validation Studies [c]
Adhesive	1
Analytical reagent	1
Chemical intermediate	6
Coatings	1
Dye	1
Hormone	3
Metabolic inhibitor	1
Natural product	7
Pesticide	9
Pesticide metabolite	1
Pharmaceutical	42
Pharmaceutical metabolite	1
Plasticizer	3
Could not be assigned to a product class	4

[a] Based on information provided in **Table 2-5**.

[b] Product classes were assigned based on information contained in *The Merck Index* and the U.S. National Library of Medicine's ChemID database.

[c] Because a substance may be assigned to more than one product class, the number of substances selected for validation studies totaled across product classes exceeds the number of selected substances.

Table 2–5: Chemical and Product Classes of the 78 Recommended Substances [a]

Substance	CASRN	Chemical Class	Product Class
Actinomycin D	50-76-0	Phenoxazone; Lactone; Peptide	Pharmaceutical
Ammonium perchlorate	7790-98-9	Organic acid; Organic salt	Pharmaceutical
Anastrazole	120511-73-1	Nitrile; Triazole	Pharmaceutical
4-Androstenedione	63-05-8	Steroid, nonphenolic	Hormone
Apigenin	520-36-5	Flavanoid; Flavone; Phenol	Natural product
Apomorphine	58-00-4	Heterocycle; Quinoline	Pharmaceutical
Atrazine	1912-24-9	Aromatic amine; Triazine; Arylamine	Pesticide
Bicalutamide	90357-06-5	Anilide; Nitrile; Sulfone	Pharmaceutical
Bisphenol A	80-05-7	Diphenylalkane; Bisphenol; Phenol	Chemical intermediate
Bisphenol B	77-40-7	Diphenylalkane; Bisphenol; Phenol	Adhesive, Chemical intermediate, Coatings
Butylbenzyl phthalate	85-68-7	Phthalate	Plasticizer
2-*sec*-Butylphenol	89-72-5	Phenol	Pharmaceutical
CGS 18320B	112808-99-8	Nitrile; Imidazole	Metabolic inhibitor
Clomiphene citrate	50-41-9	Chlorinated triphenylethylene; Benzylidene; Stilbene	Pharmaceutical
Corticosterone	50-22-6	Steroid, nonphenolic	Pharmaceutical
Coumestrol	479-13-0	Coumestan; Ketone Benzopyranone; Coumarin	Natural product
4-Cumylphenol	599-64-4	Phenol	Chemical intermediate
Cycloheximide	66-81-9	Piperidine; Glutaramide	Pharmaceutical

Table 2–5: Chemical and Product Classes of the 78 Recommended Substances [a]
(continued)

Substance	CASRN	Chemical Class	Product Class
Cyproterone acetate	427-51-0	Nitrile; Diphenyl ether; Organochlorine	Pharmaceutical
Daidzein	486-66-8	Flavanoid; Isoflavone; Phenol	Natural product
p,p'-DDE	72-55-9	Organochlorine; Diphenylalkene	Pesticide metabolite
o,p'-DDT	789-02-6	Organochlorine; Diphenylalkene	Pesticide
Dexamethasone	50-02-2	Steroid, nonphenolic	Pharmaceutical
Dibenzo[*a,h*]anthracene	53-70-3	Polycyclic aromatic hydrocarbon; Anthracene	None
Di-*n*-butyl phthalate	84-74-2	Phthalate	Plasticizer
Diethylhexyl phthalate	117-81-7	Phthalate	Plasticizer
Diethylstilbestrol	56-53-1	Stilbene; Benzylidene; Diphenylalkene	Pharmaceutical
5α-Dihydrotestosterone	521-18-6	Steroid, nonphenolic	Pharmaceutical
17α-Estradiol	57-91-0	Steroid, phenolic; Estrene	None
17β-Estradiol	50-28-2	Steroid, phenolic; Estrene	Hormone
Estrone	53-16-7	Steroid, phenolic; Estrene	Pharmaceutical
17α-Ethinyl estradiol	57-63-6	Steroid, phenolic	Pharmaceutical
Ethyl paraben	120-47-8	Paraben; Organic acid	Pharmaceutical
Fadrozole	102676-47-1	Imidazole; Nitrile	Pharmaceutical
Fenarimol	60168-88-9	Heterocycle; Pyrimidine	Pesticide
Finasteride	98319-26-7	Steroid, nonphenolic; Androstene	Pharmaceutical

Table 2–5: Chemical and Product Classes of the 78 Recommended Substances [a]
(continued)

Substance	CASRN	Chemical Class	Product Class
Flavone	525-82-6	Flavanoid; Flavone	Natural product
Fluoranthene	206-44-0	Polycyclic aromatic hydrocarbon; Fluorene	None
Fluoxymestrone	76-43-7	Steroid, nonphenolic	Pharmaceutical
Flutamide	13311-84-7	Amide; Anilide; Nitrobenzene	Pharmaceutical
Genistein	446-72-0	Flavanoid; Isoflavone; Phenol	Natural product
Haloperidol	52-86-8	Butyrophenone; Ketone; Piperazine	Pharmaceutical
meso-Hexestrol	84-16-2	Diphenylalkane; Bisphenol; Phenol	Pharmaceutical
Hydroxyflutamide	52806-53-8	Amide; Anilide; Nitrobenzene	Pharmaceutical, Metabolite
4-Hydroxytamoxifen	68047-06-3	Triphenylethylene; Phenol; Benzylidene; Stilbene	Pharmaceutical
ICI 182,780	129453-61-8	Steroid, phenolic	Pharmaceutical
Kaempferol	520-18-3	Flavanoid; Flavone; Phenol	Natural product
Kepone	143-50-0	Organochlorine; Chlorinated bridged cycloalkane	Pesticide
Ketoconazole	65277-42-1	Imidazole; Piperazine	Pharmaceutical
Linuron	330-55-2	Urea	Pesticide
Medroxyprogesterone acetate	71-58-9	Steroid, nonphenolic; Polycyclic hydrocarbon	Pharmaceutical
p, p'-Methoxychlor	72-43-5	Organochlorine; Chlorinated hydrocarbon	Pesticide
Methyl testosterone	58-18-4	Steroid, nonphenolic; Androstene	Pharmaceutical

Table 2-5: Chemical and Product Classes of the 78 Recommended Substances[a]
(continued)

Substance	CASRN	Chemical Class	Product Class
Methyltrienolone	965-93-5	Steroid, nonphenolic; Estrene	Pharmaceutical
Mifepristone	84371-65-3	Steroid, nonphenolic; Estrene	Pharmaceutical
Morin	480-16-0	Flavanoid; Flavone; Phenol	Dye
Nilutamide	63612-50-0	Heterocycle; Imidazole	Pharmaceutical
p-n-Nonylphenol	104-40-5	Alkylphenol; Phenol	Chemical intermediate
Norethynodrel	68-23-5	Steroid, nonphenolic; Norpregnene	Pharmaceutical
4-*tert*-Octylphenol	140-66-9	Alkylphenol; Phenol	Chemical intermediate
Oxazepam	604-75-1	Benzodiazepine	Pharmaceutical
Phenobarbital	57-30-7	Heterocycle; Pyrimidine	Pharmaceutical
Phenolphthalin	81-90-3	Triphenylmethane; Diphenyalkane carboxylic acid	Analytical reagent
Pimozide	2062-78-4	Piperidine; Benzimidazole	Pharmaceutical
Procymidone	32809-16-8	Organochlorine; Cyclic imide	Pesticide
Progesterone	57-83-0	Steroid, nonphenolic; Pregnenedione	Pharmaceutical
Propylthiouracil	51-52-5	Pyrimidine; Uracil	Pharmaceutical
Reserpine	50-55-5	Heterocycle; Yohimban	Pharmaceutical
Sodium azide	26628-22-8	Organic salt; Azide	
Spironolactone	52-01-7	Steroid, nonphenolic; Pregnene lactone	Pharmaceutical

Table 2-5: Chemical and Product Classes of the 78 Recommended Substances [a]
(continued)

Substance	CASRN	Chemical Class	Product Class
Tamoxifen	10540-29-1	Triphenylethylene; Benzylidene; Stilbene	Pharmaceutical
Testosterone	58-22-0	Steroid, nonphenolic	Pharmaceutical
12-*O*-Tetradecanoyl-phorbol-13-acetate	16561-29-8	Phorbol ester; Terpene	Pharmaceutical
L-Thyroxine	51-48-9	Aromatic amino acid	Hormone
17β-Trenbolone	10161-33-8	Steroid, nonphenolic; Estrene	Pharmaceutical
2,4,5-Trichloro-phenoxyacetic acid	93-76-5	Organochlorine; Chlorinated aromatic hydrocarbon	Pesticide
Vinclozolin	50471-44-8	Organochlorine; Cyclic imide; Carbamate	Pesticide
Zearalenone	17924-92-4	Resorcylic acid lactone; Phenol	Chemical intermediate, Natural product

[a] Substances were assigned to chemical and product classes based on available information from standardized references (e.g., *The Merck Index* and the U.S. National Library of Medicine's ChemID database) and from an assessment of chemical structure.

Abbreviations:
p,p′-DDE =1,1-Dichloro-2,2-di(*p*-chlorophenyl)ethylene; *o,p′*-DDT =1,1,1-Trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane; *p,p′*-DDT =1,1,1-Trichloro-2,2-di(4-chlorophenyl)ethane