Proposed Substances for Validation of Estrogen Receptor (ER) and Androgen Receptor (AR) Binding and Transcriptional Activation (TA) Assays[1]

Background

The U.S. EPA has proposed an Endocrine Disruptor Screening Program (EDSP) (Federal Register, Vol. 63, No. 248, pp. 71541-71568, December 28, 1998) to evaluate pesticides and other substances for their potential to induce hormone related health effects. The proposed EDSP consists of a Tier 1 screening battery of *in vitro* and *in vivo* test methods designed to identify substances capable of interacting with the endocrine system, and Tier 2 *in vivo* testing designed to confirm and extend the Tier 1 results. The proposed Tier 1 screening battery includes *in vitro* estrogen receptor (ER) and androgen receptor (AR) assays. Currently, the U.S. EPA proposes that either a binding assay or a transcriptional activation (TA) assay could be used to identify ER or AR activity.

Prior to implementing the EDSP, the proposed test methods must be adequately validated to determine their usefulness, limitations, and transferability. Accordingly, the U.S. EPA asked the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to assess the current validation status of the *in vitro* ER/AR binding and TA test methods. As an initial step in this process, NICEATM prepared Background Review Documents (BRD) reviewing available data and protocols for the following:

- *In vitro* ER binding assays
- *In vitro* ER TA assays
- *In vitro* AR binding assays
- *In vitro* AR TA assays

Each BRD included, for the type of assay considered:

- A compilation of all relevant published and submitted data
- Protocols submitted to NICEATM by interested scientists
- Recommended minimum procedural standards
- Recommended substances for future validation studies:
  - 33 substances for validation of ER binding assays
  - 31 and 21 substances for validation of ER TA agonism and antagonism assays, respectively
  - 31 substances for validation of AR binding assays
  - 28 and 25 substances for validation of AR TA agonism and antagonism assays, respectively

[1] Inclusion of a substance in Appendix A does not mean that the U.S. EPA, NICEATM, ICCVAM, or the Expert Panel has or will make a determination that any of the uses of the chemical will pose a significant risk. Further, this table should not be interpreted as a list of 'endocrine disruptors'; the substances listed are simply compounds which have been, or may prove to be, useful in developing, standardizing or validating screening and testing methods.

NICEATM/ICCVAM Expert Panel Evaluation

An Expert Panel meeting, organized by NICEATM and ICCVAM, was convened on May 21-22, 2002 in Research Triangle Park, NC, to assess the current validation status of these four types of *in vitro* endocrine disruptor screening methods, and to develop recommendations on:

- *In vitro* assays that should be considered for further evaluation in validation studies and their relative priority
- The adequacy of the proposed minimum procedural standards for each of the four types of assays
- The adequacy of available test method protocols for assays recommended for validation studies
- The adequacy and the appropriateness of the substances recommended for use in future validation studies

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

In terms of the adequacy and the appropriateness of the substances recommended for use in future validation studies, the Expert Panel generally agreed with the list of substances proposed by NICEATM but also recommended:

- The same substances should be tested in both binding and TA assays for each type of receptor (i.e., in ER binding/TA assays; in AR binding/TA assays)
- The proportion of negative substances should be increased to at least 25 % of the total chemicals to better evaluate assay specificity
- Clomiphene citrate, a substance with a potency two orders of magnitude lower than 17β-estradiol in ER binding assays, should be included
- Substances likely to alter reporter gene transcriptional activation via a non-receptor binding mechanism should be included (i.e., actinomycin D, cycloheximide, sodium azide, 12-O-tetradecanoylphorbol-13-acetate)
- Substances from non-represented chemical classes (e.g., phthalates, polychlorinated biphenyls) and additional substances from under-represented classes (e.g., polycyclic aromatic hydrocarbons) should be included
- A central repository of chemicals of high purity should be made available for use in future validation studies

ICCVAM EDWG and NICEATM Proposed List of Substances for Validation

To facilitate validation studies on *in vitro* ER and AR test methods, the ICCVAM Endocrine Disruptor Working Group (EDWG) and NICEATM developed a revised list of proposed substances. This consolidated list incorporates the recommendations of the Expert Panel, including the recommendation to have at least 25% negative substances in the proposed list of substances for future validation studies. Only a small number of negative substances (3 to 5) for each assay type (binding or TA) were identified and included in the BRDs. These were selected

[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 at http://iccvam.niehs.nih.gov

because they were uniformly negative in multiple studies based on available published and submitted data. Many of the substances positive for ER binding/TA assays are expected to be negative in the corresponding AR binding/TA assays. In order to increase the proportion of negative substances for each of the six types of assays, the same substances are recommended for testing in both ER and AR based assays. A common list would also limit the total number of chemicals that would need to be maintained in a chemical repository.

Candidate Substances

Initially, 122 candidate substances were identified for future validation studies; this list was subsequently reduced to 78 substances (see **Appendix A**). The 122 candidate substances included:

- The 81 substances recommended in the four BRDs for future validation studies (in the Binding Assay BRDs, see Section 12, Table 12-1; in the TA BRDs, see Section 12, Tables 12-1 and 12-2) http://iccvam.niehs.nih.gov/methods/endodocs/ed_brd.htm)
- Twenty-one of the 44 substances scheduled for testing in *in vivo* mammalian endocrine disruptor assays by the U.S. EPA and the Organisation for Economic Co-operation and Development (OECD)[3] and not already included in the list. The *in vivo* list included five substances (oxazepam, phenobarbital, and L-thyroxine, ammonium perchlorate, propylthiouracil) that are known to disrupt thyroid function. These five substances are unlikely to have ER and AR disrupting properties and thus would likely be negative in binding/TA assays.
- Nine of the 38 substances not already included in the list and scheduled for testing in *in vitro* endocrine disruptor assays by the U.S. EPA.
- The 11 additional substances recommended by the Expert Panel.
- Three substances with ER or AR activity (dibenzo[*a,h*]anthracene, fluoranthene, zearalenone) belonging to chemical classes that were previously under-represented (i.e., polycyclic aromatic hydrocarbons for the first two substances, resorcylic acid lactone/phenol for the third substance).
- Both 4-hydroxytamoxifen and tamoxifen were included in the list, because 4-hydroxytamoxifen was recommended by the Expert Panel and the salt of tamoxifen, tamoxifen citrate, is being tested *in vitro*. Although, *in vivo*, tamoxifen will be metabolized to 4-hydroxytamoxifen, this is unlikely to occur *in vitro* especially if the semi-purified ER is used (as recommended by the Expert Panel) in the ER binding assay for validation. Based on literature values, 4-hydroxytamoxifen binds with higher affinity to the ER (approximately two orders of magnitude higher) than the parent compound. In the ER TA assay, tamoxifen could be metabolized to 4-hydroxytamoxifen but this will depend on the metabolic capacity of the cell line finally chosen for ER TA. Both compounds are ER antagonists although 4-hydroxytamoxifen is more active.

[3]On July 8, 2002, NICEATM received a list of the substances selected/recommended for *in vitro* endocrine disruptor testing by the U.S. EPA and for *in vitro* and *in vivo* endocrine disruptor testing by the U.S. EPA and/or the OECD from Mr. Gary E. Timm in the U.S. EPA Office of Science Coordination and Policy, Washington, DC. This list was assembled by Mr. James Kariya for presentation at the March 2002 Meeting of the U.S. EPA Endocrine Disruptor Methods Validation Subcommittee.

Selection of 78 Proposed Substances

The 122 candidate substances were reduced to 78 proposed substances as follows:

- Methyl parathion and 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin, both proposed by the U.S. EPA for *in vivo* testing, were excluded from this list because of their high toxicity.
- Testosterone propionate, also proposed by the U.S. EPA for *in vivo* testing, was excluded because it is readily hydrolyzed *in vivo* to testosterone, which was selected for inclusion in the list.
- Three substances (arochlor 1254, 4-chloro-4'-biphenylol, 2',4',6'-trichloro-4-biphenylyol) were excluded because of hazardous waste concerns.
- The remaining list of 116 substances was reduced to 78 substances by excluding substances not scheduled for *in vitro* testing by the U.S. EPA or *in vivo* testing by the U.S. EPA and the OECD (with the exceptions noted above).

The expected performance of these 78 substances in the various *in vitro* endocrine disruptor assays is provided in **Table 1A** (*in vitro* ER assays) and **1B** (*in vitro* AR assays). Based on the available data, about 44% and 55% of the substances are expected to be negative in *in vitro* ER and AR based assays, respectively. Seventy chemical classes are included in this list of 78 chemicals; the distribution of substances among these 70 chemical classes is provided in **Table 2**. In addition, 13 product classes are represented in the list of 78 chemicals; the distribution of substances among these 13 product classes is provided in **Table 3**.

Purpose and Advantages of the Consolidated List

The purpose of this 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. The current proportion of "negative" substances in this list appears to be 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 appear to be active in both *in vitro* ER and AR assays. Thus, the actual proportion of "negative" substances is expected to be closer to 25% than the projected 40 to 50%. This consolidated list includes all but four substances recommended by the U.S. EPA and the OECD for testing in various *in vivo* endocrine disruptor assays. Three of these substances (Arochlor 1254, methyl parathion, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) were not included because of their toxicity and/or hazardous waste disposal concerns. The fourth substance (letrozole) was not included because there is a possibility that it will not be tested *in vivo*.

The current goal of the U.S. EPA is to validate *in vitro* ER/AR binding and TA assays as components of a Tier 1 screening battery that includes both *in vitro* and *in vivo* test methods. The purpose of the *in vitro* assays in this screening battery is not to predict biologically relevant *in vivo* responses, but rather to provide mechanistic information that will be considered in conjunction with *in vivo* test results in a weight-of-evidence evaluation. For this intended use, it therefore might not be essential to characterize responses for the entire set of substances expected to produce negative results (e.g., thyroid disruptors, aromatase inhibitors). However, to fully characterize the usefulness and limitations of a battery of *in vitro* tests methods for predicting *in vivo* responses, all of the 78 substances are recommended for testing in each of the

in vitro assays. This data will allow comparison of *in vitro* and *in vivo* data, including assessment of the predictive value of *in vitro* test batteries for *in vivo* effects. The generation of both *in vivo* and *in vitro* data will facilitate the future development of more predictive *in vitro* endocrine disruptor assays.

Table 1. Distribution of Anticipated Responses in *In Vitro* ER/AR Binding and TA Assays*

A. *In Vitro* ER Assays

Expected Response	ER Binding	ER TA**				
		Agonist A	Agonist B	Antagonist A	Antagonist B	Agonist and Antagonist
Positive	44 (56%)	13 (17%)	9 (12%)	0 (0%)	5 (6%)	4 (5%)
Negative	34 (44%)	34 (44%)				
Unknown		13 (17%)				
Total	78	78				

B. *In Vitro* AR Assays

Expected Response	AR Binding	AR TA**				
		Agonist A	Agonist B	Antagonist A	Antagonist B	Agonist and Antagonist
Positive	35 (45%)	6 (8%)	4 (5%)	1 (1%)	9 (12%)	8 (10%)
Negative	43 (55%)	43 (55%)				
Unknown		7 (9%)				
Total	78	78				

*Based on information provided in Appendix A.

**TA Classifications: Substances classified as unknown have not been tested in either agonist or antagonist assays; Agonist A = substances classified as agonist that have not been tested for antagonist activity; Agonist B = substances classified as agonist that have tested negative for antagonist activity; Antagonist A = substances classified as antagonist that have not been tested for agonist activity; Antagonist B = substances classified as antagonist that have tested negative for agonist activity; Agonist and Antagonist = substances that have tested positive in both assays. For purposes of this data presentation, substances classified as "+/-" are classified as positive.

Table 2. Distribution of Selected and Non-Selected Substances Among Various Chemical Classes*

Chemical Class[a]	No. of Substances Selected for Validation Studies	No. of Substances Not Selected for Validation Studies
Acyclic acid		1
Alkylphenol	2	1
Amide	2	1
Androstene	2	2
Anilide	3	
Anthracene	1	
Aromatic amine	1	
Aromatic amino acid	1	
Arylamine	1	
Azide	1	
Benzidine		1
Benzimidazole	1	
Benzodiazepine	1	
Benzopyran		2
Benzopyranone	1	
Benzylidene	3	1
Bisphenol	3	1
Butyrophenone	1	
Carbamate	1	1
Chalcone		1
Chlorinated aromatic hydrocarbon	1	
Chlorinated bridged cycloalkane	1	2
Chlorinated cyclodiene		1
Chlorinated hydrocarbon	1	
Chlorinated cycloalkane		1
Chlorinated triphenylethylene	1	

Chemical Class[a]	No. of Substances Selected for Validation Studies	No. of Substances Not Selected for Validation Studies
Chlorobenzene		2
Coumarin	1	
Coumestan	1	
Cyclic hydrocarbon		1
Cyclic imide	2	
Dioxin		1
Diphenolalkane	3	1
Diphenylalkene	3	1
Diphenyalkane carboxylic acid	1	
Diphenyl ether	1	
Estrene	6	2
Flavanoid	6	6
Flavanone		1
Flavone	4	1
Fluorene	1	
Glutaramide	1	
Heterocycle	5	
Hydroxylated biphenyl		1
Hydroxy polychlorinated biphenyl		1
Imidazole	4	
Indene		1
Isoflavone	2	3
Ketone	2	
Lactone	1	
Nitrile	5	2
Nitrobenzene	2	
Norpregnane		1
Norpregnene	1	

Chemical Class[a]	No. of Substances Selected for Validation Studies	No. of Substances Not Selected for Validation Studies
Organic acid	2	
Organic salt	2	
Organochlorine	8	7
Organothiophosphate		2
Paraben	1	
Peptide	1	
Phenol	14	6
Phenoxazone	1	
Phorbol ester	1	
Phthalate	3	
Piperazine	2	
Piperidine	2	1
Polychlorinated biphenyl		1
Polycyclic aromatic hydrocarbon	2	
Polycyclic hydrocarbon	1	
Pregnenedione	1	3
Pregnadiene		1
Pregnane		1
Pregnene lactone	1	
Pyrimidine	3	
Quinoline	1	
Resorcylic acid lactone	1	2
Steroid, nonphenolic	15	8
Steroid, phenolic	5	5
Stilbene	3	5
Sulfone	1	
Terpene	1	
Triazine	1	1

Chemical Class[a]	No. of Substances Selected for Validation Studies	No. of Substances Not Selected for Validation Studies
Triazole	1	1
Triphenylethylene	2	4
Triphenylmethane	1	
Uracil	1	
Urea	1	
Yohimban	1	

*Based on information provided in Appendix B.

[a]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.

Note: As a substance may be included in more than one chemical class, the number of substances totaled across chemical classes exceeds the number of substances in the list.

Table 3. Distribution of Selected and Non-Selected Substances Among Various Product Classes*

Product Class[a]	No. of Substances Selected for Validation Studies	No. of Substances Not Selected for Validation Studies
Adhesive	1	
Analytical reagent	1	
Antioxidant		1
Chemical intermediate	6	3
Coatings	1	
Dielectric fluid		1
Dye	1	
Hormone	3	
Lubricant additive		1
Metabolic inhibitor	1	
Natural product	7	7
Pesticide	9	10
Pesticide metabolite	1	
Pharmaceutical	42	22
Pharmaceutical metabolite	1	
Plasticizer	3	
None could be assigned	4	5

*****Based on information provided in Appendix B.

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

Note: As a substance may be assigned to more than one product class, the number of substances totaled across product classes exceeds the number of substances in the list.

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Substance	CAS RN	Proposed for Validation Efforts	Anticipated *In Vitro* Response		*In Vitro* Data (NICEATM)[a]			Studies Proposed by OECD	Studies Proposed by the U.S. EPA				Comments
			ER TA and/or Binding	AR TA and/or Binding	Binding[b]	Transcriptional Activation							
						Agonism[c]	Antagonism[d]	Uterotrophic (U)/ Hershberger (H)/ 407[e]	*In vitro* Binding[f]	Pubertal Assays (PA) and Intact Male (IM)[g]	*In utero* through Lactation[h]	One or Two Generation or FRS[i]	
Substances Proposed for Validation													
Actinomycin D	50-76-0	†	-	-									inhibits protein synthesis; recommended by the Expert Panel
Ammonium perchlorate	7790-98-9	†	-	-							**		thyroid disruptor; being considered for testing *in vivo* by U.S. EPA
Anastrazole	120511-73-1	†	-	-		AR-				IM			aromatase inhibitor; being tested *in vivo* by U.S. EPA
4-Androstenedione	63-05-8	†	+	+	ER+/AR+++	ER-/AR+++			AR				strong AR agonist; being tested *in vitro* by U.S. EPA
Apigenin	520-36-5	†	+	-	ER+++	ER+++	ER#-				**		strong ER agonist; being considered for testing *in vivo* by U.S. EPA
Apomorphine	58-00-4	†	-	-						IM			dopamine D1/D2 receptor agonist; being tested *in vivo* by U.S. EPA
Atrazine	1912-24-9	†	+	+	ER+/AR+	ER-/AR-	ER-/AR-		AR	M-PA	**		binds weakly to AR and ER; being tested *in vitro* and *in vivo* by U.S. EPA
Bicalutamide	90357-06-5	†	-	+	AR+++	AR+	AR##						AR antagonist; recommended by the Expert Panel
Bisphenol A	80-05-7	†	+	+	ER++	ER+/AR-	ER-/AR#-	U	ER	F-PA			weak ER agonist; being tested *in vitro* and *in vivo* by U.S. EPA and *in vivo* by OECD
Bisphenol B	77-40-7	†	+	-	ER++	ER++/AR-			ER				ER agonist; being tested *in vitro* by U.S. EPA
Butylbenzyl phthalate	85-68-7	†	+	-	ER±	ER++/AR-	ER-/AR-				**		ER agonist; being considered for testing *in vivo* by U.S. EPA
2-*sec* -Butylphenol	89-72-5	†	+	-	ER+				ER				binds weakly to ER; being tested *in vitro* by U.S. EPA
CGS 18320B	112808-99-8	†	-	-				407					aromatase inhibitor; being tested *in vivo* by OECD
Clomiphene citrate	50-41-9	†	+	-	ER++				ER				binds to ER; being tested *in vitro* by U.S. EPA; recommended by the Expert Panel
Corticosterone	50-22-6	†	-	+	ER-/AR+	ER-/AR-			ER/AR				binds weakly to AR; being tested *in vitro* by U.S. EPA
Coumestrol	479-13-0	†	+	-	ER+++	ER++/AR-	ER-		ER	IM			ER agonist; being tested *in vitro* and *in vivo* by U.S. EPA
4-Cumylphenol	599-64-4	†	+	-		ER+/AR-			ER				weak ER agonist; being tested *in vitro* by U.S. EPA
Cycloheximide	66-81-9	†	-	-									inhibits protein synthesis; recommended by the Expert Panel as a negative control
Cyproterone acetate	427-51-0	†	-	+	AR+++	ER-/AR+	AR##		AR	IM			AR agonist and antagonist; being tested *in vitro* and *in vivo* by U.S. EPA
Daidzein	486-66-8	†	+	-	ER++	ER+	ER-		ER				weak ER agonist; being tested *in vitro* by U.S. EPA
p,p' -DDE**	72-55-9	†	+	+	ER±/AR++	ER+/AR±	ER-/AR#-	H/407	AR	M-PA; IM			weak AR agonist and antagonist; being tested *in vitro* and *in vivo* by U.S. EPA and *in vivo* by OECD
o,p' -DDT**	789-02-6	†	+	+	ER++/AR+	ER+/AR-	ER#/AR#	U					weak ER agonist and antagonist; weak AR antagonist; being tested *in vivo* by OECD

Substance	CAS RN	Proposed for Validation Efforts	Anticipated *In Vitro* Response		*In Vitro* Data (NICEATM)[a]			Studies Proposed by OECD	Studies Proposed by the U.S. EPA				Comments
						Transcriptional Activation							
			ER TA and/or Binding	AR TA and/or Binding	Binding[b]	Agonism[c]	Antagonism[d]	Uterotrophic (U)/ Hershberger (H)/ 407[e]	*In vitro* Binding[f]	Pubertal Assays (PA) and Intact Male (IM)[g]	*In utero* through Lactation[h]	One or Two Generation or FRS[i]	
Dexamethasone	50-02-2	†	+	+	ER-/AR-	ER±/AR+			AR				weak ER and AR agonist; being tested *in vitro* by U.S. EPA
Dibenzo[*a,h*]anthracene	53-70-3	†	+	+	ER-	ER-/AR+	ER##						ER antagonist; included as it belongs to an under-represented class of substances
Di-*n* -butyl phthalate	84-74-2	†	+	-	ER±	ER+/AR-	ER-	U		M-PA		1G	ER agonist; being tested *in vivo* by U.S. EPA and OECD
Diethylhexyl phthalate	117-81-7	†	-	-	ER-	AR-			AR				being tested *in vitro* by U.S. EPA
Diethylstilbestrol	56-53-1	†	+	+	ER+++/AR++	ER+++/AR-	AR#				**		ER agonist; being considered for testing *in vivo* by U.S. EPA
5α-Dihydrotestosterone****	521-18-6	†	+	+	ER++/AR+++	ER+/AR+++		H	AR				weak ER agonist; strong AR agonist; being tested *in vitro* by U.S. EPA and *in vivo* by OECD
17α-Estradiol	57-91-0	†	+	-	ER+++	ER++/AR-			ER				ER agonist; being tested *in vitro* by U.S. EPA
17β-Estradiol****	50-28-2	†	+	+	ER+++/AR++	ER+++/AR++	AR##		ER/AR	IM	**	FRS	strong ER agonist; AR agonist and antagonist; being tested *in vitro* and *in vivo* by U.S. EPA
Estrone	53-16-7	†	+	+	ER+++/AR++	ER+++/AR++			ER				strong ER agonist; AR agonist; being tested *in vitro* by U.S. EPA
17α-Ethinyl estradiol	57-63-6	†	+	+	ER+++/AR++	ER+++/AR-		U/407	ER	F-PA			strong ER agonist; being tested *in vitro* and *in vivo* by U.S. EPA and *in vivo* by OECD
Ethyl-4-hydroxybenzoate	120-47-8	†	-	-					ER				being tested *in vitro* by U.S. EPA
Fadrozole	102676-47-1	†	-	-						F-PA; IM		FRS	aromatase inhibitor; being tested *in vivo* by U.S. EPA
Fenarimol	60168-88-9	†	+	-		ER+	ER#			F-PA			aromatase inhibitor; being tested *in vivo* by U.S. EPA
Finasteride	98319-26-7	†	-	-		AR-	AR-	H		M-PA; IM			5α-reductase inhibitor; being tested *in vivo* by U.S. EPA and by OECD
Flavone	525-82-6	†	+	-	ER-	ER±	ER###			M-PA; IM			ER antagonist; being tested *in vivo* by U.S. EPA
Fluoranthene	206-44-0	†	-	+	ER-	ER-	ER-/AR#						AR antagonist; included as it belongs to an under-represented class of substances
Fluoxymestrone	76-43-7	†	-	+	AR++	AR+	AR-						weak AR agonist; recommended by the Expert Panel
Flutamide	13311-84-7	†	-	+	AR++	ER-/AR-	AR#	H/407		M-PA; IM		FRS	AR antagonist; being tested *in vivo* by U.S. EPA and by OECD
Genistein	446-72-0	†	+	-	ER++	ER+	ER#	U/407					weak ER agonist and antagonist; being tested *in vivo* by OECD
Haloperidol	52-86-8	†	-	-						IM			dopamine D2 receptor antagonist; being tested *in vivo* by U.S. EPA
meso -Hexestrol	84-16-2	†	+	-	ER+++	ER+++			ER				strong ER agonist; being tested *in vitro* by U.S. EPA
Hydroxyflutamide	52806-53-8	†	+	+	ER±/AR++	AR+	AR##						AR agonist and antagonist; recommended by the Expert Panel
4-Hydroxytamoxifen	68047-06-3	†	+	-	ER+++	ER±/AR-	ER###						ER antagonist; recommended by the Expert Panel
ICI 182,780	129453-61-8	†	+	-	ER+++	ER-/AR-	ER###/AR-			IM			ER antagonist; being tested *in vivo* by U.S. EPA
Kaempferol	520-18-3	†	+	-	ER++	ER+	ER-		ER				weak ER agonist; being tested *in vitro* by U.S. EPA

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						Transcriptional Activation							
			ER TA and/or Binding	AR TA and/or Binding	Binding[b]	Agonism[c]	Antagonism[d]	Uterotrophic (U)/ Hershberger (H)/ 407[e]	*In vitro* Binding[f]	Pubertal Assays (PA) and Intact Male (IM)[g]	*In utero* through Lactation[h]	One or Two Generation or FRS[i]	
Kepone	143-50-0	†	+	+	ER++/AR++	ER+/AR-	AR#-						binds to ER and AR; included as it belongs to an under-represented class of substances
Ketoconazole	65277-42-1	†	-	+		AR±	AR-			F and M-PA; IM			weak AR agonist; being tested *in vivo* by U.S. EPA
Linuron	330-55-2	†	-	+	AR+	ER-/AR+	AR#	H	AR	M-PA			weak AR agonist and antagonist; being tested *in vitro* and *in vivo* by U.S. EPA and *in vivo* by OECD
Medroxyprogesterone acetate	71-58-9	†	-	+	AR+++	AR+			AR				weak AR agonist; being tested *in vitro* by U.S. EPA
Methoxychlor	72-43-5	†	+	+	ER+/AR+	ER+/AR-	ER-/AR#	U	AR	F and M-PA; IM	**	2G (avian)/FRS	weak ER agonist; AR antagonist; being tested *in vitro* and *in vivo* by U.S. EPA and *in vivo* by OECD
Methyl testosterone	58-18-4	†	+	+	AR+++	ER++/AR++		H/407		M-PA	**	FRS	ER and AR agonist; being tested *in vivo* by U.S. EPA and by OECD
Methyltrienolone	965-93-5	†	-	+	AR+++	ER-/AR+	AR-		AR				weak AR agonist; being tested *in vitro* by U.S. EPA
Mifepristone	84371-65-3	†	-	+	AR+++	ER-/AR++	AR###			IM			AR agonist and antagonist; being tested *in vivo* by U.S. EPA
Morin	480-16-0	†	+	-	ER+				ER				binds weakly to ER; being tested *in vitro* by U.S. EPA
Nilutamide	63612-50-0	†	-	+	AR+++	AR±	AR##						AR antagonist; recommended by the Expert Panel
n -Nonylphenol	104-40-5	†	+	+	ER++	ER++/AR±	ER#/AR###	U/407	ER				ER agonist and antagonist; AR antagonist: being tested *in vitro* by U.S. EPA and *in vivo* by OECD
Norethynodrel	68-23-5	†	+	-	ER++				ER				binds to ER; being tested *in vitro* by U.S. EPA
4 -tert -Octylphenol	140-66-9	†	+	-	ER++	ER++/AR-			AR				ER agonist; being tested *in vitro* by U.S. EPA
Oxazepam	604-75-1	†	-	-						IM			enhances thyroid hormone excretion; being tested *in vivo* by U.S. EPA
Phenobarbital	57-30-7	†	-	-		ER-/AR-				F and M-PA; IM			enhances thyroid hormone excretion; being tested *in vivo* by U.S. EPA
Phenolphthalin	81-90-3	†	+	-	ER+				ER				binds weakly to ER; being tested *in vitro* by U.S. EPA
Pimozide	2062-78-4	†	-	-						F and M-PA			dopamine receptor antagonist; being tested *in vivo* by U.S. EPA
Procymidone	32809-16-8	†	-	+	AR+	ER-/AR-	AR#	H	AR				AR antagonist; being tested *in vitro* by U.S. EPA and *in vivo* by OECD
Progesterone	57-83-0	†	+	+	ER+/AR+++	ER±/AR+	ER-/AR#-		ER/AR	IM			AR agonist; being tested *in vitro* and *in vivo* by U.S. EPA
Propylthiouracil	51-52-5	†	-	-				407		F and M-PA; IM	**	2G	inhibits T3/T4 synthesis; being tested *in vivo* by U.S. EPA and by OECD
Reserpine	50-55-5	†	-	-						IM			depletes dopamine; being tested *in vivo* by U.S. EPA
Sodium azide	26628-22-8	†	-	-									cytotoxicant; recommended by the Expert Panel as a negative control
Spironolactone	52-01-7	†	-	+	AR+++	AR+	AR##		AR				AR agonist and antagonist; being tested *in vitro* by U.S. EPA
Tamoxifen	10540-29-1	†	+	-	ER+++	ER±/AR-	ER###						ER antagonist; tamoxifen citrate is being tested *in vitro* by U.S. EPA
Testosterone	58-22-0	†	+	+	ER±/AR+++	ER±/AR+++	AR-		AR	IM			strong AR agonist; being tested *in vitro* and *in vivo* by U.S. EPA

Substance	CAS RN	Proposed for Validation Efforts	Anticipated *In Vitro* Response		*In Vitro* Data (NICEATM)[a]			Studies Proposed by OECD	Studies Proposed by the U.S. EPA				Comments
			ER TA and/or Binding	AR TA and/or Binding	Binding[b]	Transcriptional Activation							
						Agonism[c]	Antagonism[d]	Uterotrophic (U)/ Hershberger (H)/ 407[e]	*In vitro* Binding[f]	Pubertal Assays (PA) and Intact Male (IM)[g]	*In utero* through Lactation[h]	One or Two Generation or FRS[i]	
12-*O* -Tetradecanoylphorbol-13-acetate	16561-29-8	†	-	-									activates ligand independent cell division; recommended by the Expert Panel
L-Thyroxine	51-48-9	†	-	-				407					thyroid hormone; being tested *in vivo* by OECD
17β-Trenbolone	10161-33-8	†	-	+	AR+++	ER-		H					binds strongly to the AR; being tested *in vivo* by OECD
2,4,5-Trichlorophenoxyacetic acid	93-76-5	†	+	-	ER-	ER+			ER				weak ER agonist; being tested *in vitro* by U.S. EPA
Vinclozolin	50471-44-8	†	+	+	ER±/AR++	ER-/AR-	AR###	H	AR	M-PA; IM	**	1G/FRS	AR antagonist; being tested *in vitro* and *in vivo* by U.S. EPA and *in vivo* by OECD
Zearalenone	17924-92-4	†	+	-	ER+++	ER++/AR-	ER#-						ER agonist; included as it belongs to an under-represented class of substances
Substances Considered but not Included for Validation													
Arochlor 1254	11097-69-1		-	-	ER-	ER-					**		does not bind to ER; omitted due to disposal concerns
Bendiocarb	22781-23-3		+	-		ER-	ER#						ER antagonist but no *in vivo* testing planned
Bisphenol C 2	14868-03-2		+	-	ER+++								binds strongly to the ER but no *in vivo* testing planned
4-*tert* -Butylphenol	98-54-4		+	-	ER±	ER+							weak ER agonist but no *in vivo* testing planned
Chlordane	57-74-9		+	-	ER-	ER+							weak ER agonist but no *in vivo* testing planned
4-Chloro-4'-biphenylol	28034-99-3		+	-	ER+	ER+	ER-						weak ER agonist but no *in vivo* testing planned; concern regarding disposal
Cortisol	50-23-7		-	+	ER-/AR-	ER-/AR++							AR agonist but no *in vivo* testing planned
Cyanoketone	4248-66-2		-	-	AR-								negative for ER and AR binding and no *in vivo* testing planned
p,p' -DDT**	50-29-3		+	+	ER+/AR+	ER+/AR-	AR##						weak ER agonist; AR antagonist; but no *in vivo* testing planned
Dicofol	115-32-2		+	-		ER+/AR-	ER-						weak ER agonist but no *in vivo* testing planned
Droloxifene	82413-20-5		+	-	ER+++	ER±	ER###						ER antagonist but no *in vivo* testing planned
Equol	531-95-3		+	-	ER++	ER+/AR-							ER agonist but no *in vivo* testing planned
Estriol	50-27-1		+	+	ER+++/AR-	ER++							ER agonist but no *in vivo* testing planned
Fenitrothion	122-14-5		-	+		AR+	AR#-						weak AR agonist but no *in vivo* testing planned
Formononetin	485-72-3		+	-	ER±	ER+	ER#						weak ER agonist; ER antagonist but no *in vivo* testing planned
Genistin	529-59-9		+	-	ER±	ER±	ER-						weak ER agonist but no *in vivo* testing planned
Heptachlor	76-44-8		-	-	ER-	ER-							does not bind to ER and no *in vivo* testing planned
4-Hydroxyandrostenedione	566-48-3		-	+	AR++								binds to AR but no *in vivo* testing planned
17α-Hydroxyprogesterone	68-96-2		-	+	AR++	ER-							binds to AR but no *in vivo* testing planned

Substance	CAS RN	Proposed for Validation Efforts	Anticipated *In Vitro* Response		*In Vitro* Data (NICEATM)[a]			Studies Proposed by OECD	Studies Proposed by the U.S. EPA				Comments
						Transcriptional Activation							
			ER TA and/or Binding	AR TA and/or Binding	Binding[b]	Agonism[c]	Antagonism[d]	Uterotrophic (U)/ Hershberger (H)/ 407[e]	*In vitro* Binding[f]	Pubertal Assays (PA) and Intact Male (IM)[g]	*In utero* through Lactation[h]	One or Two Generation or FRS[i]	
Hydroxytoremifene	110503-62-3		+	-		ER±	ER###						ER antagonist but no *in vivo* testing planned
ICI 164,384	98007-99-9		+	-	ER+++	ER±	ER###						ER antagonist but no *in vivo* testing planned
Kaempferide	491-54-3		+	-		ER±	ER###						ER antagonist but no *in vivo* testing planned
Letrozole	112809-51-5		-	-						F-PA (?)			aromatase inhibitor; questionable whether letrozole will be tested *in vivo*
Levonorgestrel	797-63-7		+	+	AR+++	ER±/AR+++							weak ER agonist; strong AR agonist; but no *in vivo* testing planned
Lindane	58-89-9		+	-	ER±	ER+/AR-	AR-						weak ER agonist but no *in vivo* testing planned
Melengestrol acetate	2919-66-6		+	+	AR++	ER+							weak ER agonist but no *in vivo* testing planned
Mestranol	72-33-3		+	-	ER++	ER+							weak ER agonist but no *in vivo* testing planned
Methyl parathion	298-00-0		+	-		ER+						2G avian	decreases LH in quail; weak ER agonist; being tested *in vivo* by EPA, but not considered as it is highly toxic
Mirex	2385-85-5		-	-	ER-	ER-/AR-	AR-						does not bind to ER or AR and no *in vivo* testing planned
Nafoxidine	1845-11-0		+	-	ER++	ER±/AR-							binds to ER but no *in vivo* testing planned
Naringenin	480-41-1		+	-	ER+	ER+	ER#-						weak ER agonist but no *in vivo* testing planned
19-Nortestosterone	434-22-0		+	+	ER++/AR+++	ER±/AR+++							weak ER agonist; AR agonist; but no *in vivo* testing planned
4-Octylphenol	1806-26-4		+	-	ER+	ER+	ER#						weak ER agonist; ER antagonist but no *in vivo* testing planned
Phloretin	60-82-2		+	-	ER++	ER+	ER#-						weak ER agonist but no *in vivo* testing planned
Pregnenolone	145-13-1	†	-	+	AR±								binds weakly to AR but no *in vivo* testing planned
Raloxifene	84449-90-1		+	-	ER+++	ER-	ER###						ER antagonist but no *in vivo* testing planned
Simazine	122-34-9		+	-	ER-	ER±	ER-						weak ER agonist and no *in vivo* testing planned
β-Sitosterol	83-46-5		+	-	ER-	ER±/AR-							weak ER agonist and no *in vivo* testing planned
Tamoxifen citrate	54965-24-1		+	-	ER+++	ER-	ER#		ER				ER antagonist; tamoxifen selected instead of tamoxifen citrate as the citrate will hydrolyze *in vivo;* thus, for *in vitro* assays tamoxifen is more appropriate; being tested *in vitro* by U.S. EPA but no *in vivo* testing planned
Testosterone propionate	57-85-2		-	-				H					no binding or TA data; administered as propionate *in vivo* due to solubility; hydrolyzes *in vivo* to testosterone, thus for *in vitro* assays testosterone is more appropriate; being tested by OECD *in vivo*
2,3,7,8, -Tetrachlorodibenzo-*p* -dioxin	1746-01-6		+	+	ER-	ER++/AR-	ER###/AR#				**		ER agonist and antagonist; not considered due to extreme toxicity
2',4',6',-Trichloro-4-biphenylyol	14962-28-8		+	-	ER+++	ER+	ER#-						weak ER agonist; no *in vivo* testing planned; concern regarding disposal

Substance	CAS RN	Proposed for Validation Efforts	Anticipated *In Vitro* Response		*In Vitro* Data (NICEATM)[a]			Studies Proposed by OECD	Studies Proposed by the U.S. EPA				Comments
						Transcriptional Activation							
			ER TA and/or Binding	AR TA and/or Binding	Binding[b]	Agonism[c]	Antagonism[d]	Uterotrophic (U)/ Hershberger (H)/ 407[e]	*In vitro* Binding[f]	Pubertal Assays (PA) and Intact Male (IM)[g]	*In utero* through Lactation[h]	One or Two Generation or FRS[i]	
α-Zearalanol	26538-44-3		+	-	ER+++	ER++/AR-	ER##						ER agonist and antagonist; no *in vivo* testing planned
β-Zearalenol	71030-11-0		+	-	ER+++	ER+							ER agonist; no *in vivo* testing planned

*Inclusion of a substance in this table does not mean that U.S. EPA or NICEATM/ICCVAM Expert Panel has or will make a determination that any of the uses of the chemical will pose a significant risk. Further, this table should not be taken as a list of endocrine disruptors; the substances listed are simply compounds, which have, or may prove to be, useful in developing, standardizing or validating screening and testing methods.

Empty cells indicate that no relevant data were identified and no validation tests are planned for that substance in that particular assay.

***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

*** Indicates that a particular substance has been recommended for testing in the *in vivo* assay through lactation.

**** 17β-Estradiol is the recommended positive control substance for the ER binding and ER TA assays; for AR binding, 5α Dihydrotestosterone is the recommended positive control if a purified AR protein is used, while Methyltrienolone or Mibolerone is recommended if intact cells, or cytosol is used. For AR TA assays, either 5α Dihydrotestosterone or Methyltrienolone is recommended as the positive control.

† Indicates that a substances has been recommended for testing.

[a] *In vitro* data obtained from the literature or from reports submitted to NICEATM and summarized in four Background Review Documents (ER Binding, ER TA, AR Binding, AR TA); reports available at http://iccvam.niehs.nih.gov/methods/endodocs/ed_brd.htm.

[b] +++ Indicates that the substance was relatively active as measured by the relative binding affinity (RBA) (RBA value was >1); ++ indicates that the substance was moderately active (RBA value was between 1 and 0.01); + indicates that the substance was weakly active (RBA value was < than 0.01); - indicates that an $IC50_x$alue was not obtained and thus an RBA value could not be determined; ± indicates an equivocal response (i.e., in different studies, the substance was reported as positive and negative). The inhibitory concentration 50 ($IC5_{0)}$ is the concentration of test substances that displaces 50% of the radiolabeled reference estrogen or androgen from the receptor.

c +++ Indicates that the substance was relatively active (EC_{50} value was <0.001); ++ indicates that the substance was moderately active (EC_{50} value was between 0.001 and 0.1); + indicates that the substance was weakly active (EC_{50} value was >0), or a positive response was reported without an EC_{50} value. The EC_{50} is the effective concentration that displaces 50% of the radiolabeled reference estrogen or androgen from the receptor.

[d] ### Indicates that the substance was uniformly positive in multiple assays; ## indicates that the substance was positive in the majority of assays in which it was tested; # indicates that the substance was positive in the single assay in which it was tested; #- indicates the substance was positive in one assay but was also negative in one or more assays; - indicates that the substance was uniformly negative in multiple assays.

[e] In the Uterotrophic Assay, the weight of the uterus is determined after exposure of the rat or mouse to the test substance for three days. In the Hershberger assay, sex accessory gland weights are determined in castrated male rats 4-7 days after treatment of the animals with the test substance (agonistic response) or the test substance with testosterone (antagonistic response). The 407 protocol assess the effect on all organs including the reproductive organs of varying concentrations of the test substance administered daily to 7 week-old female rats for 28 days. After treatment, the estrus cycle is evaluated by daily vaginal smears for 5 days while treatment is continued. Thyroid hormone levels are measured. All the reproductive tissues are fixed for histology. All the reproductive tissues from the males are fixed for histology. Sperm from one epididymis is enumerated and examined morphologically.

[f] Indicates which substances are proposed for testing by the U.S. EPA for ER and AR receptor binding.

[g] In the Intact male assay (IM), adult male rats (70-90 days of age) are dosed daily by intraperitoneal injection for 14 days and sacrificed 24 hours after the last dose. The testes, epididymes, seminal vesicles and prostate are weighed. One cauda epididymis is weighed and the sperm found in this cauda are evaluated for motility and concentration. One testis, epididymis, and thyroid gland are fixed for histological evaluation. Blood hormone levels are measured. This assay detects effects on male reproductive organs that are sensitive to antiandrogens and substances that interfere with testosterone biosynthesis. The male Pubertal assay (M-PA) measures the age of preputial separation (PPS). Androgens accelerate and antiandrogens and estrogens delay PPS. Animals are dosed daily by gavage beginning one week before puberty (40 days of age). The rats are sacrificed and all the reproductive tissues are weighed. Hisptopathological analysis of the thyroid is performed and blood levels of the thyroid hormone are measured. In the female (F), the Pubertal Assay (F-PA) measures the time it takes for the vaginal opening to be observed following single or multiple daily treatments from 21 days of age (weaning).

[g] The *in utero* through lactation assay has been recommended, but the U.S. EPA has not made a decision for its further development or for validation.

[g] FRS = Fish Reproductive Screen; 1G = one generation; 2G = two generation

Substance	CAS RN	Chemical Class[a]	Product Class[b]
Substances Proposed for Validation			
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	Diphenolalkane; Bisphenol; Phenol	Chemical intermediate
Bisphenol B	77-40-7	Diphenolalkane; 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; Benzopyranone; Coumarin; Ketone	Natural product
4-Cumylphenol	599-64-4	Phenol	Chemical intermediate
Cycloheximide	66-81-9	Piperidine; Glutaramide	Pharmaceutical
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

Substance	CAS RN	Chemical Class[a]	Product Class[b]
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-4-hydroxybenzoate	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
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
Hexestrol	84-16-2	Diphenolalkane; Bisphenol; Phenol	Pharmaceutical
Hydroxyflutamide	52806-53-8	Amide; Anilide; Nitrobenzene	Pharmaceutical, Metabolite

Substance	CAS RN	Chemical Class[a]	Product Class[b]
4-Hydroxytamoxifen	68047-06-3	Triphenylethylene; Phenol	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
Methoxychlor	72-43-5	Organochlorine; Chlorinated hydrocarbon	Pesticide
Methyl testosterone	58-18-4	Steroid, nonphenolic; Androstene	Pharmaceutical
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 -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

Substance	CAS RN	Chemical Class[a]	Product Class[b]
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
Tamoxifen	10540-29-1	Triphenylethylene; Benzylidene; Stilbene	Pharmaceutical
Testosterone	58-22-0	Steroid, nonphenolic	Pharmaceutical
12-*O* -Tetradecanoylphorbol-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-Trichlorophenoxyacetic 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

Substance	CAS RN	Chemical Class[a]	Product Class[b]
Substances Considered but not Included for Validation			
Arochlor 1254	11097-69-1	Polychlorinated biphenyl	Dielectric fluid
Bendiocarb	22781-23-3	Acyclic acid; Carbamate	Pesticide
Bisphenol C 2	14868-03-2	Diphenolalkane; Bisphenol; Phenol	Chemical intermediate
4-*tert* -Butylphenol	98-54-4	Phenol	Chemical intermediate, Lubricant additive, Antioxidant
Chlordane	57-74-9	Organochlorine; Chlorinated bridged cycloalkene	Pesticide
4-Chloro-4'-biphenylol	28034-99-3	Organochlorine; Hydroxylated biphenyl	None
Cortisol	50-23-7	Steroid, phenolic; Pregnenedione	Pharmaceutical
Cyanoketone	4248-66-2	Steroid, phenolic; Androstene; Nitrile	Pharmaceutical
p,p' -DDT†	50-29-3	Organochlorine; Diphenylalkene	Pesticide
Dicofol	115-32-2	Cyclic hydrocarbon; Chlorobenzene	Pesticide
Droloxifene	82413-20-5	Triphenylethylene; Stilbene	Pharmaceutical
Equol	531-95-3	Flavanoid; Isoflavone; Benzopyran	Pharmaceutical
Estriol	50-27-1	Steroid, phenolic; Estrene	Pharmaceutical
Fenitrothion	122-14-5	Organothiophosphate	Pesticide
Formononetin	485-72-3	Flavanoid; Isoflavone; Phenol	Pharmaceutical, Natural product
Genistin	529-59-9	Flavanoid; Isoflavone	Natural product
Heptachlor	76-44-8	Chlorinated cyclodiene; Indene	Pesticide
4-Hydroxyandrostenedione	566-48-3	Steroid, phenolic; Androstene	Pharmaceutical

Substance	CAS RN	Chemical Class[a]	Product Class[b]
17α-Hydroxyprogesterone	68-96-2	Steroid, nonphenolic; Pregnedione	Pharmaceutical
Hydroxytoremifene	110503-62-3	Triphenylethylene; Stilbene	None
ICI 164,384	98007-99-9	Steroid, phenolic; Amide	Pharmaceutical
Kaempferide	491-54-3	Flavanoid; Flavone	Natural product
Letrozole	112809-51-5	Triazole; Nitrile	Pharmaceutical
Levonorgestrel	797-63-7	Steroid, nonphenolic; Pregnane	Pharmaceutical
Lindane	58-89-9	Organochlorine; Chlorinated cycloalkane	Pesticide, Pharmaceutical
Melengestrol acetate	2919-66-6	Steroid, nonphenolic; Pregnadiene	Pharmaceutical
Mestranol	72-33-3	Steroid, nonphenolic; Norpregnane	Pharmaceutical
Methyl parathion	298-00-0	Organothiophosphate	Pesticide
Mirex	2385-85-5	Organochlorine; Chlorinated bridged cycloalkene	Pesticide, Pharmaceutical
Nafoxidine	1845-11-0	Stilbene; Triphenylethylene	Pharmaceutical
Naringenin	480-41-1	Flavanoid; Flavanone; Benzopyran	None
19-Nortestosterone	434-22-0	Steroid, nonphenolic; Estrene	Pharmaceutical
4-Octylphenol	1806-26-4	Alkylphenol; Phenol	Chemical intermediate
Phloretin	60-82-2	Flavanoid; Chalcone, Phenol	Natural product
Pregnenolone	145-13-1	Steroid, nonphenolic; Pregnenedione	Pharmaceutical
Raloxifene	84449-90-1	Benzidine; Stilbene; Piperidine	Pharmaceutical
Simazine	122-34-9	Organochlorine; Triazine	Pesticide

Substance	CAS RN	Chemical Class[a]	Product Class[b]
β-Sitosterol	83-46-5	Steroid, nonphenolic; Androstene	Natural product, Pharmaceutical
Tamoxifen citrate	54965-24-1	Triphenylethylene; Stilbene; Benzylidene	Pharmaceutical
Testosterone propionate	57-85-2	Steroid, nonphenolic	Pharmaceutical
2,3,7,8, -Tetrachlorodibenzo-*p* -dioxin	1746-01-6	Dioxin; Chlorobenzene	None
2',4',6',-Trichloro-4-biphenylyol	14962-28-8	Organochlorine; Hydroxy polychlorinated biphenyl	None
α-Zearalanol	26538-44-3	Resorcylic acid lactone; Phenol	Natural product
β-Zearalenol	71030-11-0	Resorcylic acid lactone	Natural product

**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

[a]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.

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

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