

## **Addendum to**

**ICCVAM Evaluation of *In Vitro* Test Methods for  
Detecting Potential Endocrine Disruptors:  
Estrogen Receptor and Androgen Receptor Binding and  
Transcriptional Activation Assays  
(NIH Publication No: 03-4503)**

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## List of Abbreviations and Acronyms

Antag.	Antagonist
AR	Androgen receptor
BG-1	Ovarian carcinoma derived cells
BRD	Background Review Document
CASRN	Chemical Abstracts Service Registry Number
DDE	1,1-Dichloro-bis[4-chlorophenyl]ethylene
DDT	Dichlorodiphenyltrichloroethane
Dept.	Department
DHT	5 $\alpha$ -Dihydrotestosterone
DMSO	Dimethyl sulfoxide
EC <sub>50</sub>	Half-maximal effective concentration
ED	Endocrine Disruptor
EDSP	Endocrine Disruptor Screening Program
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee
EDWG	Endocrine Disruptor Working Group
EPA	U.S. Environmental Protection Agency
ER	Estrogen receptor
<i>FR</i>	<i>Federal Register</i>
IC <sub>50</sub>	Concentration of a test substance inhibiting the reference estrogen or androgen response by 50%
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
JME	Japanese Ministry of the Environment
K <sub>i</sub>	Equilibrium dissociation constant of a receptor-ligand
K <sub>ow</sub>	Octanol/water partition coefficient
LUMI-CELL <sup>®</sup>	Chemical-activated luciferase expression assay
MeSH	U.S. National Library of Medicine's Medical Subject Headings
Min.	Minimum
mg	Milligram

mL	Milliliter
mM	Millimolar
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyl
P.L.	Public Law
pM	Picomolar
pmol	Picomole
RBA	Relative binding affinity
RNA	Ribonucleic Acid
TA	Transcriptional activation
$\mu$ M	Micromolar



## PREFACE

In April 2000, the U.S. Environmental Protection Agency (EPA) asked the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to evaluate the validation status of *in vitro* estrogen receptor (ER) and androgen receptor (AR) binding and transcriptional activation (TA) test methods, which were proposed as possible components of the EPA Endocrine Disruptor Screening Program (EDSP) (EPA 1998). Because a large number of *in vitro* ER- and AR-based test methods were known to exist, it was expected that at least some of these would have been adequately validated and could, following a review of existing data and verification of their validity, be included in the EDSP. The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) subsequently compiled available data and information on *in vitro* ER and AR binding and TA test methods. Four Background Review Documents (BRDs) were produced that provided comprehensive reviews of the available data for each of the four types of test methods.

In collaboration with ICCVAM and the ICCVAM Endocrine Disruptor Working Group (EDWG), NICEATM organized an independent evaluation of these *in vitro* test methods for detecting substances with potential endocrine disrupting activity on May 20-21, 2002 in Research Triangle Park, NC. This meeting was open to the public with time set aside for public comment. The 24-member scientific expert panel (Panel) reviewed the information and recommendations provided in the four draft BRDs and concluded that there were no adequately validated *in vitro* ER- or AR-based test methods. The Panel provided recommendations on the following:

- specific test methods that should undergo further evaluation in validation studies and their relative priority for evaluation
- the adequacy of proposed minimum procedural standards
- the adequacy of protocols for specific test methods recommended for validation
- the adequacy and appropriateness of reference substances proposed for validation studies

In October, 2002, NICEATM published:

- 1) the Panel's report (ICCVAM 2002e)
- 2) a *Federal Register (FR)* notice requesting public comment on the Panel's report (*FR* Vol. 67, No. 204, pp. 64902-64903, October 22, 2002)

ICCVAM considered the Panel's conclusions and recommendations and public comments received in response to the *FR* notice. ICCVAM then developed test method recommendations that included minimum procedural standards and a list of 78 reference substances that should be used to standardize and validate *in vitro* ER and AR binding and TA test methods. ICCVAM's conclusions and recommendations, as well as the final BRDs and other supporting information, were made publicly available in May of 2003 in the report: *ICCVAM Evaluation of the In Vitro Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays* (ICCVAM 2003).

NICEATM recently assessed the commercial availability and cost for the 78 substances recommended by ICCVAM for use in *in vitro* ER and AR binding and TA validation studies. During this assessment, NICEATM identified three substances that are not commercially available, one substance with limited commercial availability, and six substances that were considered relatively expensive. ICCVAM considered this information and subsequently proposed revisions to the original list. In March, 2006, NICTEAM published a *FR* notice requesting public comment on these ICCVAM recommendations (*FR* Vol. 71, No. 51, pp. 13597-13598, March 16, 2006). ICCVAM recommended replacement of those substances that are not commercially available or have limited commercial availability with commercially available substances that have similar ER or AR binding or agonist TA activity profiles, or are similarly concordant for antagonist TA activity across studies. Four of the six more expensive substances were recommended for retention because of chemophysical properties that were considered unique. The two remaining expensive substances were recommended for replacement with less expensive substances that have similar ER or AR binding or agonist TA activity profiles, or that are similarly concordant for antagonist TA activity across studies. No public comments were received in response to the proposed

changes. ICCVAM subsequently approved the proposed changes to the list of 78 ICCVAM recommended substances. The changes are described in this addendum to the report:

*ICCVAM Evaluation of In Vitro Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays* (ICCVAM 2003).

The availability of this updated list of recommended reference substances should facilitate standardization and validation of *in vitro* endocrine disruptor test methods. Data generated from adequately validated and accepted *in vitro* and *in vivo* Tier 1 screening assays will be used to reach weight-of-evidence decisions on whether to conduct large multi-generational Tier 2 *in vivo* studies. It is also anticipated that data obtained during the validation of the four different types of *in vitro* ER- and AR-based test methods will help characterize the extent to which individual or batteries of *in vitro* endocrine disruptor test methods might be used to further reduce the expected requirements for animal use in Tier I screening.

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## EXECUTIVE SUMMARY

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) has revised the original list of 78 substances recommended for the validation of *in vitro* estrogen receptor (ER) and androgen receptor (AR) binding and transcriptional activation (TA) test methods, based on an assessment of their commercial availability and cost. Six of the original substances have been removed and replaced with substances considered to have similar activity. Three substances were not commercially available and the commercial availability of another substance was restricted. ICCVAM has replaced those four substances with commercially available substances that have similar ER or AR binding or agonist TA activity profiles, or are similarly concordant for antagonist TA activity across studies. Six substances were identified that were considered relatively expensive. Four of the six expensive substances were retained because their chemico-physical properties were considered unique. The two other substances have been replaced with less expensive substances that have similar ER or AR binding or agonist TA activity profiles, or are similarly concordant for antagonist TA activity across studies.

## 1.0 INTRODUCTION

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recommended a list of 78 substances to use for the validation of *in vitro* estrogen receptor (ER) and androgen receptor (AR) binding and transcriptional activation (TA) test methods (ICCVAM 2003). A number of factors and criteria were considered by ICCVAM in compiling this list, including assay data and recommendations provided in Background Review Documents (BRDs) on ER and AR binding and TA test methods (ICCVAM 2002a, b, c, d), and in the ICCVAM Endocrine Disruptor Expert Review Panel Final Report (ICCVAM 2002e). To allow for a direct comparison between results obtained from *in vitro* and *in vivo* ED test methods, the list also includes substances proposed for *in vivo* ED testing by the U.S. Environmental Protection Agency (EPA) and the Organisation for Economic Co-operation and Development (OECD)<sup>1</sup>. These factors and considerations are discussed in detail in the report: *ICCVAM Evaluation of the In Vitro Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays* (referred to below as the ICCVAM ED Test Method Evaluation Document) (ICCVAM 2003).

Two practical criteria for reference substances recommended for validation studies are that the substances should be: 1) commercially available, and 2) to the extent possible, reasonably priced. The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) recently assessed the commercial availability and pricing information for the original list of 78 recommended substances and, based on the information obtained, ICCVAM has revised the recommended list based on these criteria. The purpose of this addendum to the 2003 ICCVAM ED Test Method Evaluation Document (ICCVAM 2003) is to:

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<sup>1</sup> 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 vivo* endocrine disruptor testing by the EPA or the OECD from Mr. Gary 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 ED Methods Validation Subcommittee. On August 4, 2005, NICEATM received an updated list of substances for *in vitro* and *in vivo* endocrine disruptor testing by the EPA from Mr. Timm.

1. Recommend specific replacements for those substances that are not commercially available, can only be purchased in a limited amount, or are considered to be relatively expensive (i.e., would cost more than \$2000 per 500 mg, the minimum amount that a laboratory might need to test the substance as part of a validation study)
2. Review the criteria used to select the original substances for *in vitro* ER and AR binding and TA validation studies, which were also used by ICCVAM in selecting the replacements

## **2.0 SELECTION OF THE ORIGINAL ICCVAM RECOMMENDED REFERENCE SUBSTANCES FOR VALIDATION OF *IN VITRO* ENDOCRINE DISRUPTOR TEST METHODS**

In its 2003 report, ICCVAM recommended 78 substances that should be used for validation of *in vitro* ER and AR binding and TA assays. This section reviews the basis for the selection of these 78 substances.

### **2.1 Background Review Documents**

In February 2002, four draft BRDs were published that documented available data for ER and AR binding and TA test methods for detecting endocrine disruptors:

- Current Status of Test Methods for Detecting Endocrine Disruptors: *In Vitro* Estrogen Receptor Binding Assays (ICCVAM 2002a) (referred to below as the ER Binding BRD)
- Current Status of Test Methods for Detecting Endocrine Disruptors: *In Vitro* Estrogen Receptor Transcriptional Activation Assays (ICCVAM 2002b) (referred to below as the ER TA BRD)
- Current Status of Test Methods for Detecting Endocrine Disruptors: *In Vitro* Androgen Receptor Binding Assays (ICCVAM 2002c) (referred to below as the AR Binding BRD)

- Current Status of Test Methods for Detecting Endocrine Disruptors: *In Vitro* Androgen Receptor Transcriptional Activation Assays (ICCVAM 2002d) (referred to below as the AR TA BRD)

Each draft BRD included a list of proposed reference substances recommended for future validation studies of the test method considered. 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
- the distribution of the proposed substances among chemical classes

## 2.2 ICCVAM Endocrine Disruptor Expert Panel Recommendations

An Expert Panel (Panel) met in June 2002 and developed recommendations on the adequacy and appropriateness of the substances proposed in the draft BRDs for use in future validation studies. The 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 both binding and TA agonism and antagonism test methods
- 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
- 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

## 2.3 Development of the Original ICCVAM Recommended Reference Substances

In late 2002, ICCVAM reviewed the Panel's recommendations regarding substances that should be used in future validation studies and developed a final list of recommended reference substances. To meet the Panel's recommendation that at least 25% of the substances proposed for validation studies be negative for binding or TA for the receptor being used, an assumption was made that substances positive in ER binding or TA test methods would likely be negative in the corresponding AR-based test methods and vice versa, and that such substances could serve as presumptive negatives in the alternative receptor-based test methods. This approach would also minimize the total number of different substances that would be needed to validate ER/AR test methods.

### 2.3.1 Selection of the Original Candidate Substances

Initially, 122 substances were identified as candidate reference substances for validation studies (**Appendix A**). This list of candidates consisted of:

- 85 substances recommended in the four draft BRDs for future validation studies (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)
- 44 substances scheduled for testing in *in vivo* mammalian endocrine disruptor test methods by the EPA and the OECD; 22 of which were included in the lists mentioned above. Adding the remaining 22 substances increased the total number of candidate substances to 107. 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 validation studies.
- 38 substances scheduled for testing in *in vitro* endocrine disruptor test methods by the EPA, 29 of which were included in the BRD lists above. Adding the additional nine substances increased the total number of candidate substances to 116.



- The Panel specifically recommended six additional substances (actinomycin D, bicalutamide, cycloheximide, hydroxyflutamide, sodium azide, and 12-O-tetradecanoylphorbol-13-acetate), resulting in a total of 122 candidate substances.

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 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[4chlorophenyl] 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 the Environment (JME).

### 2.3.2 Selection of the Original 78 Recommended 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 the 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 test methods

- 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 test methods

The remaining list of 114 candidate substances was reduced to 78 substances (**Appendix A**) by excluding substances not scheduled for *in vitro* testing by the EPA or for *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 the EPA. The number of substances recommended in the draft BRDs for the validation of *in vitro* ER and AR binding and TA assays and their expected performance in the various *in vitro* endocrine disruptor test methods are provided in **Table 1**. **Table 2** contains the expected performance for *in vitro* ER-based test methods while **Table 3** contains similar information for *in vitro* AR-based test methods.

**Table 1** Candidate Substances in the Draft BRDs for the Validation of *In Vitro* ER and AR Binding and TA Assays<sup>a</sup>

<i>In Vitro</i> Assay Type	Number of Substances	Number of Positive Substances	Number of Negative Substances
ER Binding	33	30 (91%)	3 (9%)
ER TA Agonist	31	26 (84%)	5 (16%)
ER TA Antagonist	21	17 (81%)	4 (19%)
AR Binding	31	28 (90%)	3 (10%)
AR TA Agonist	28	18 (64%)	10 (36%)
AR TA Antagonist	25	21 (84%)	4 (16%)

<sup>a</sup>Based on information provided in BRDs on ER and AR binding and TA test methods (ICCVAM 2002a,b,c,d).

**Table 2 Distribution of Anticipated Responses of the Original 78 Recommended Test Substances in *In Vitro* ER Binding and TA Assays<sup>a</sup>**

Expected Response	ER Binding	ER TA	
		Agonist	Antagonist
Positive <sup>b</sup> and Presumed Positive <sup>c</sup>	41 (53%)	35 (45%)	11 (14%)
Negative <sup>d</sup> and Presumed Negative <sup>e</sup>	37 (47%)	43 (55%)	67 (86%)
Total	78	78	78

<sup>a</sup>Based on information provided in Sections 3.0 through 6.0 of the ICCVAM ED Test Method Evaluation Document (NIH Publication No: 03-4503). Counts include the recommended reference estrogen, 17 $\beta$ -estradiol.

<sup>b</sup>Represents substances for which ER binding or TA data are available, which indicate a positive response in the respective test method (i.e., substances tested in more than one study that were positive in > 50% of the studies).

<sup>c</sup>Represents substances that were positive in  $\leq$  50% of reported studies; that were positive but tested in only one study; or that have no relevant receptor binding or TA data available for the respective test method but which are presumed positive based on their known mechanism of action or their responses in other endocrine disruptor screening test methods (e.g., methyl testosterone, an ER agonist, is presumed positive in ER binding assays).

<sup>d</sup>Represents substances that tested negative for ER binding or ER TA in multiple studies, when tested up to the limit dose

<sup>e</sup>Represents substances which are presumed negative based on the available data, their known mechanism of action, or their responses in other endocrine disruptor screening test methods (e.g., anastrozole and fadrozole, known aromatase inhibitors, are presumed negative in ER binding and TA assays).

**Table 3** Distribution of Anticipated Responses of the 78 Recommended Test Substances in *In Vitro* AR Binding and TA Assays<sup>a</sup>

Expected Response	AR Binding	AR TA	
		Agonist	Antagonist
Positive <sup>b</sup> and Presumed Positive <sup>c</sup>	34 (44%)	22 (28%)	21 (27%)
Negative <sup>d</sup>	44 (56%)	56 (72%)	57 (73%)
Total	78	78	78

<sup>a</sup> Based on information provided in Sections 3.0 through 6.0 of the ICCVAM ED Test Method Evaluation Document (NIH Publication No: 03-4503). Counts include the recommended reference androgen, methyltrienolone.

<sup>b</sup> Represents substances for which receptor binding or TA data are available, which indicate a positive response in the respective test method (i.e., substances tested in more than one study that were positive in > 50% of the studies).

<sup>c</sup> Represents substances that were positive in  $\leq 50\%$  of reported studies; that were positive but tested in only one study; or that have no relevant receptor binding or TA data available for the respective test method but which are presumed positive based on their known mechanism of action or their responses in other endocrine disruptor screening test methods (e.g., ketoconazole, an AR agonist, is presumed positive in AR binding assays).

<sup>d</sup> Represents 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., anastrozole 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 recommended limit dose of 1 mM.

Based on the available data, approximately 47% and 56% of the substances are expected to be negative in *in vitro* ER- and AR-based test methods, respectively.

### 2.3.3 Purpose and Advantages of the Original List of 78 Substances

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 test methods are adequately characterized across a broad range of chemical classes and responses. The current goal of the EPA is to validate *in vitro* ER and AR binding and TA test methods as components of the Endocrine Disruptor Screening Program (EDSP) Tier 1 screening battery, which includes both *in vitro* and *in vivo* test methods. This list includes most of the substances proposed for the validation of Tier 1 and Tier 2 *in vivo* test methods, which will help characterize the usefulness and limitations of the Tier 1 screening battery for prioritizing substances for Tier 2 testing, and hopefully facilitate development of more predictive *in vitro* endocrine disruptor test methods. 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 test methods have been reported as active in both.

#### 2.3.4 Original Minimum Lists of Substances for Validation of *In Vitro* Endocrine Disruptor Test Methods

Because the purpose of these *in vitro* test methods 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 used for the validation of *in vitro* ER and AR binding and TA test methods. For each receptor type, the same substances are proposed for testing in binding and TA (agonist and antagonist) studies. This approach will allow for a direct comparison of the reliability and performance of these different types of *in vitro* endocrine disruptor test methods. The substances proposed in the draft BRDs and those being tested by the EPA in *in vitro* studies have been used as the foundation for each minimum list. The additional substances recommended by the Panel and those likely to be negative for the endpoint being assessed, complete the list. The minimum lists contain 53 substances for ER binding and TA studies and 44 substances for AR binding and TA studies, with similar distributions of substances across the ranges of responsiveness and chemical classes as contained in the list of 78 substances. The original 53 and 44 substances selected for the minimum lists are provided in **Appendix A. Tables 4 and 5** show the distribution of positives and negatives for each type of assay for the minimum lists recommended substances.

**Table 4 Distribution of Anticipated Responses of the Original 53 Recommended Minimum Test Substances in *In Vitro* ER Binding and TA Assays<sup>a</sup>**

Expected Response	ER Binding	ER TA	
		Agonist	Antagonist
Positive <sup>b</sup> and Presumed Positive <sup>c</sup>	40 (75%)	34 (64%)	11 (21%)
Negative <sup>d</sup> and Presumed Negative <sup>e</sup>	13 (25%)	19 (36%)	42 (79%)
Total	53	53	53

<sup>a</sup>Based on information provided in Sections 3.0 through 6.0 of the ICCVAM ED Test Method Evaluation Document (NIH Publication No: 03-4503). Counts include the recommended reference estrogen, 17 $\beta$ -estradiol.

<sup>b</sup>Represents substances for which ER binding or TA data are available, which indicate a positive response in the respective test method (i.e., substances tested in more than one study that were positive in > 50% of the studies).

<sup>c</sup>Represents substances that were positive in  $\leq$  50% of reported studies; that were positive but tested in only one study; or that have no relevant receptor binding or TA data available for the respective test method but which are presumed positive based on their known mechanism of action or their responses in other endocrine disruptor screening test methods (e.g., methyl testosterone, an ER agonist, is presumed positive in ER binding assays).

<sup>d</sup>Represents substances that tested negative for ER binding or ER TA in multiple studies, when tested up to the limit dose

<sup>e</sup>Represents substances which are presumed negative based on the available data, their known mechanism of action, or their responses in other endocrine disruptor screening test methods (e.g., anastrozole and fadrozole, known aromatase inhibitors, are presumed negative in ER binding and TA assays).

**Table 5** Distribution of Anticipated Responses of the 44 Recommended Minimum Test Substances in *In Vitro* AR Binding and TA Assays<sup>a</sup>

Expected Response	AR Binding	AR TA	
		Agonist	Antagonist
Positive <sup>b</sup> and Presumed Positive <sup>c</sup>	33 (75%)	20 (45%)	20 (45%)
Negative <sup>d</sup>	11 (25%)	24 (55%)	24 (55%)
Total	44	44	44

<sup>a</sup> Based on information provided in Sections 3.0 through 6.0 of the ICCVAM ED Test Method Evaluation Document (NIH Publication No: 03-4503). Counts include the recommended reference androgen, methyltrienolone.

<sup>b</sup> Represents substances for which receptor binding or TA data are available, which indicate a positive response in the respective test method (i.e., substances tested in more than one study that were positive in > 50% of the studies).

<sup>c</sup> Represents substances that were positive in ≤ 50% of reported studies; that were positive but tested in only one study; or that have no relevant receptor binding or TA data available for the respective test method but which are presumed positive based on their known mechanism of action or their responses in other endocrine disruptor screening test methods (e.g., ketoconazole, an AR agonist, is presumed positive in AR binding assays).

<sup>d</sup> Represents 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., anastrozole 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 recommended limit dose of 1 mM.

### 2.3.5 Data Supporting the Original Recommended Substances

Of the 78 substances included in the original list, relevant quantitative data from *in vitro* ER and AR binding and TA studies was not available for all substances. The number and percentage of the total list of substances for which ER and AR quantitative binding and quantitative or qualitative TA data were available are presented in **Table 6**.

**Table 6** Number of Substances from Total List of 78 for AR and ER Binding and TA Test Methods for Which Relevant Quantitative or Qualitative Data was Identified<sup>a</sup>

	AR Binding	AR-TA		ER Binding	ER-TA	
		Agonist	Antagonist		Agonist	Antagonist
Number of Substances	33 <sup>b</sup>	45 <sup>c</sup>	27 <sup>c</sup>	45 <sup>b</sup>	45 <sup>c</sup>	18 <sup>c</sup>
Percentage of Total	42%	58%	35%	58%	58%	23%

<sup>a</sup> Based on information provided in Sections 3.0 through 6.0 of the ICCVAM ED Test Method Evaluation Document (NIH Publication No: 03-4503).

<sup>b</sup> The number of substances for which relevant quantitative data for *in vitro* binding studies was available.

<sup>c</sup> The number of substances for which relevant quantitative or qualitative data from agonist and antagonist studies was available.

Many of these substances were tested in only one or two of the four types of test methods 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 test methods 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. ICCVAM continues to update the database for the revised list of recommended substances.

### **3.0 REVISED ICCVAM REFERENCE SUBSTANCE LIST FOR THE VALIDATION OF *IN VITRO* ESTROGEN RECEPTOR AND ANDROGEN RECEPTOR BINDING AND TRANSCRIPTIONAL ACTIVATION TEST METHODS**

#### **3.1 Commercial Availability**

NICEATM assessed the commercial availability for the complete original list of 78 recommended substances. This assessment indicated that anastrozole, CGS18320B, and fadrozole are not commercially available and that the commercial availability of ICI 182,780 continues to be restricted to the purchase of 100 mg/year/institution. All four substances



have been removed from the recommended list of validation substances and replaced with substances that are readily available from commercial sources. The four replacements and the rationale for their selection are provided in **Section 3.3**.

### 3.2 Cost Assessment

NICEATM assessed current costs for the 74 commercially available substances on the original list. The practical consideration of reasonable pricing was based on the price for 500 mg of substance<sup>2</sup>, the expected minimum amount required per laboratory to conduct an *in vitro* ER or AR binding or TA validation study. The cost per 500 mg of substance ranged from \$7.80 to \$15,500 and all but six of the 74 substances were priced at less than \$2000. Reasonable pricing for the minimum amount of substance required to conduct an *in vitro* ER or AR binding or TA validation study was defined as a substance costing less than \$2,000 per laboratory. Based on this definition, actinomycin D (\$2,285), zearalenone (\$2,760), hydroxyflutamide (\$2,940), 4-hydroxytamoxifen (\$5,270), 12-*O*-tetradecanoylphorbol-13-acetate (\$11,220), and methyltrienolone (\$15,500) did not meet reasonable pricing criteria. Zearalone and methyltrienolone were replaced with two other comparable substances as discussed in **Section 3.3**. The other four substances were retained and the rationale for their retention is provided below.

Actinomycin D was retained as a reference substance despite its cost as it is the only RNA synthesis inhibitor (Gorski et al. 1975; Kersten and Kersten 1974; Vilee et al. 1975) on the current list of 78 reference substances. It was also included in the original minimum substance list for both ER and AR validation studies. Hydroxyflutamide also has been retained as an ED reference substance because:

- 1) it was specifically recommended by the Panel
- 2) its AR activity is well documented in the scientific literature
- 3) it was originally listed as a minimum substance to be tested for both ER and AR validation studies.

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<sup>2</sup> 500 mg was determined to be a sufficient amount of chemical per laboratory for agonist and antagonist studies, with triplicate wells run on three separate occasions following a range-finder test, and assuming that testing will be conducted to a limit dose of 1 mM.

12-*O*-tetradecanoylphorbol-13-acetate has been retained because:

- 1) it is the only phorbol ester on the list of 78 recommended substances
- 2) it has mitogenic activity that is not mediated via an ER-dependent pathway (Bamberger et al. 1998; Darne et al. 1998; Gagne et al. 1994; Martin et al. 1995; Whitman et al. 1989)
- 3) it was originally listed as a minimum substance to be tested for both ER and AR validation studies.

4-hydroxytamoxifen has been retained because:

- 1) it is the active metabolite of tamoxifen and is therefore active in all cell based systems
- 2) it is well represented in the scientific literature
- 3) it was originally listed as a minimum substance to be tested for both ER and AR validation studies

### **3.3 Basis for Selection of the Six Replacement Substances**

The replacements for the six substances that are not currently commercially available, are available only in limited quantities, or are classified as relatively expensive (with the exceptions noted) were chosen based primarily on the similarity of ER or AR binding or agonist TA activity profiles, or on similar concordance for antagonist TA activity across studies to those reference substances recommended for replacement. Activity profiles for substances were either derived from quantitative ER and AR relative binding affinity (RBA) data, or from quantitative ER and AR TA agonist half maximal effective dose (EC<sub>50</sub>) data.

Substances were classified for binding as follows:

- strongly active (RBA value was >1, designated as +++)
- moderately active (RBA value was between 1 and 0.01, designated as ++)
- weakly active (RBA value was < than 0.01, and designated as +)
- negative (designated as -) when an RBA value could not be determined

Substances were classified for ER and AR TA agonism as follows:

- strongly active ( $EC_{50}$  value was  $<0.001 \mu\text{M}$ , designated by +++)
- moderately active ( $EC_{50}$  value was between  $0.001$  and  $0.1 \mu\text{M}$ , designated by ++)
- weakly active ( $EC_{50}$  value was  $>0.1 \mu\text{M}$ , designated by +)
- negative (designated by -) when no agonist activity could be detected

Due to a lack of quantitative ER and AR TA antagonist activity data, substances were classified for ER and AR TA antagonism as follows:

- uniformly active in multiple assays (designated as ###)
- active in the majority of assays in which it was tested (designated as ##)
- active in the single assay in which it was tested (designated as #)
- found uniformly negative in all assays in which it was tested (designated as -)

Secondary considerations for replacements were for substances classified as “Substances Considered but not Included for Validation” from the original list of 122 or for substances being evaluated for ED activity by the EPA. A review of the current scientific literature was also conducted to identify replacements for substances for which a good fit for activity could not be made with substances from the original list of 122 or the substances being tested by the EPA.

The six ED reference substances that were replaced and their replacements include (see also **Tables 7 and 8**):

- Three of the six substances that were replaced are aromatase inhibitors (anastrozole, CGS 18320B, and fadrozole) with presumed negative ER and AR activity profiles. The replacements for these three substances are also aromatase inhibitors (4-OH androstenedione, chrysin, and dicofol) and are also presumed to have negative ER and AR activity profiles. 4-OH androstenedione and dicofol are classified as “Substances Considered but not Included for Validation” on the original list of 122 substances. Chrysin and dicofol are being evaluated for ED activity by the EPA.
- The replacement for ICI 182,780 as the reference standard for ER TA antagonist test methods is raloxifene, a “Substance Considered but not

Included for Validation” on the original list of 122 substances. Although raloxifene may act as an agonist in some *in vitro* systems, it is also classified as a potent Type 1 partial ER antagonist (MacGregor and Jordan 1998) with IC<sub>50</sub> values in the nanomolar range in a variety of ER TA and cell replication test methods. Although ICI 182,780 is classified as a Type 2 pure antagonist (binding prevents nuclear uptake of ER) (MacGregor and Jordan 1998), the compound is only a marginally more potent antagonist than raloxifene in similar ER TA and cell replication test methods with

**Table 7 ED Reference Substances that are Not Commercially Available versus Their Replacement Substances**

Status	*Substance	Action	Original Min. <sup>a</sup> List for ER	Original Min. <sup>a</sup> List for AR	EPA/OECD <i>In Vivo</i> Testing <sup>b</sup>	ER Binding Activity <sup>c</sup>	ER Agonist Activity <sup>d</sup>	ER Antag. <sup>e,f</sup>	AR Binding Activity <sup>c</sup>	AR Agonist Activity <sup>d</sup>	AR Antag. <sup>e,f</sup>	Total Cost Per 500 mg
Original	Anastrozole	Aromatase Inhibitor			IM					-		Not Commercially Available
Replacement	*4-OH Androstenedione	Aromatase Inhibitor			AROM	+	-		+++			\$53
Original	CGS 18320B	Aromatase Inhibitor			407							Not Commercially Available
Replacement	Chrysin	Aromatase Inhibitor			AROM							\$60
Original	Fadrozole	Aromatase Inhibitor	yes	yes	F-PA; FRS; IM							Not Commercially Available
Replacement	*Dicofol	Aromatase Inhibitor			AROM		+	-				\$88
Original	ICI 182,780	ER Antagonist	yes		IM	+++	-	###				Limited to 100 mg/yr
Replacement	*Raloxifene HCl <sup>f</sup>	ER Antagonist				+++	-	###				\$235

\* on original ICCVAM list of 122.

<sup>a</sup> Min. = Minimum

<sup>b</sup> 407 = 407 protocol of the Uterotrophic Assay, in this 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 assesses the effect on all organs including the reproductive organs, of varying concentrations of the test substance administered daily to seven 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; AROM = The EPA Placental Aromatase Assay; F-PA = Female Pubertal Assay. The 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); FRS = Fish Reproductive Screen; IM = The Intact Male Assay, in this assay, 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.

<sup>c</sup>+++ Indicates that the substance was strongly 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 RBA value could not be determined.

<sup>d</sup>+++ Indicates that the substance was strongly active (half maximal effective dose [EC<sub>50</sub>] value was <0.001 μM); ++ indicates that the substance was moderately active (EC<sub>50</sub> value was between 0.001 and 0.1 μM); + indicates that the substance was weakly active (EC<sub>50</sub> value was >0.1 μM).

<sup>e</sup> Antag. is Antagonist

<sup>f</sup>### 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 that the substance was uniformly negative in all assays.

<sup>g</sup> Note, Raloxifene may act as an agonist in some *in vitro* systems.

**Table 8 ED Reference Substances Where Total Cost Per Laboratory is in Excess of \$2000 versus Their Replacement Substances**

Status	*Substance	Action	Original Min. <sup>a</sup> List for ER	Original Min. <sup>a</sup> List for AR	EPA/OECD <i>In Vivo</i> Testing <sup>b</sup>	ER Binding Activity <sup>c</sup>	ER Agonist Activity <sup>d</sup>	ER Antag. <sup>e,f</sup>	AR Binding Activity <sup>c</sup>	AR Agonist Activity <sup>d</sup>	AR Antag. <sup>e,f</sup>	Total Cost Per 500 mg
Original	Methyltrienolone	AR Agonist		yes			-		+++	+++		\$15,500
Replacement	*19-Nortestosterone	AR Agonist				++	+/-		+++	+++		\$90
Original	Zearalenone	ER Agonist.	yes			+++	++	#		-		\$2,760
Replacement	Resveratrol	ER Agonist.				+	++	#			#	\$226

\* on original ICCVAM list of 122.

<sup>a</sup> Min. = Minimum

<sup>b</sup> None of these substances have been indicated as being evaluated for ED activity by the EPA or OECD.

<sup>c</sup>+++ Indicates that the substance was strongly 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 RBA value could not be determined; ± indicates an equivocal response (i.e., in different studies, the substance was reported as positive and negative).

<sup>d</sup>+++ Indicates that the substance was strongly active (half maximal effective concentration [EC<sub>50</sub>] value was <0.001 μM); ++ indicates that the substance was moderately active (EC<sub>50</sub> value was between 0.001 and 0.1 μM); + indicates that the substance was weakly active (EC<sub>50</sub> value was >0.1 μM); +/- indicates that the substance was weakly active or negative in different assays.

<sup>e</sup> Antag. is Antagonist

<sup>f</sup>### indicates that the substance was uniformly positive in multiple assays; ## indicates that the substance was positive in the majority of studies in which it was tested; (#) indicates that the substance was positive in the single assay in which it was tested; - indicates that the substance was uniformly negative in all assays.

IC<sub>50</sub> values in the high picomolar to low nanomolar range (Maggiolini et al. 2004; Stygar et al. 2003; Wijayarathne et al. 1999; Wilson et al. 2004; Yamamoto et al. 2005). When raloxifene is included with a weak antagonist (e.g., flavone, which is four orders of magnitude less potent than raloxifene) as positive controls, the range of potential ER activity is well bracketed by controls.

- The replacement for methyltrienolone is 19-nortestosterone. Both substances are relatively active (EC<sub>50</sub> values are <0.001 μM) and 19-nortestosterone is classified as a “Substance Considered but not Included for Validation” on the original list of 122 substances.
- The replacement for zearalenone is resveratrol. Both substances are moderately active (EC<sub>50</sub> values are between 0.001 μM and 0.1 μM) for ER agonism. The ER activity for resveratrol was acquired through a search of the scientific literature (Bhat et al. 2001; Gao et al. 2004; Klinge et al. 2005; Li et al. 2004).

Substances being replaced are indicated by bolded text in **Appendix A** and their replacements are indicated in bolded and italicized text.

The revised ICCVAM list of reference substances is provided in **Appendices B-1 through B-6**. The revised lists are sorted by ER binding activity (**Appendix B-1**), ER TA agonist activity (**Appendix B-2**), ER TA antagonism (**Appendix B-3**), AR binding activity (**Appendix B-4**), AR TA agonist activity (**Appendix B-5**), and AR TA antagonism (**Appendix B-6**) for each substance. Also listed in each appendix is relevant information about each substance (e.g., chemical class, chemical/physical properties, cost).

#### **4.0 CHEMICAL CLASS INFORMATION FOR THE REVISED ICCVAM REFERENCE SUBSTANCE LIST**

The chemical classes assigned to each reference substance in the original list (ICCVAM 2003) were revised based on a chemical classification system consistent with the U.S. National



Library of Medicine's Medical Subject Headings (MeSH; Available: <http://www.nlm.nih.gov/mesh>), an internationally recognized standardized classification scheme. This scheme was used to ensure consistency in classifying substances by chemical class among all test methods considered by ICCVAM. For ER binding and TA test methods, the distribution of substances by chemical class, as well as the distribution within each chemical class by relative activity for ER and AR binding, agonist TA activity profiles, and concordance for antagonist TA activity across studies are provided for all 78 reference substances in **Table 9** and for the minimum list of 53 substances in **Table 10**. The corresponding information for AR binding and TA test methods are provided for all 78 reference substances in **Table 11** and for the minimum list of 44 substances in **Table 12**.

**Table 9 Revised ICCVAM List of 78 Reference Substances For Validation of *In Vitro* Estrogenic Receptor Binding and Transcriptional Activation Test Methods – Distribution of Substances by Chemical Class and Available Activity Data**

MeSH <sup>4</sup> Chemical Classes	Distribution of Substances <sup>5</sup>	Binding <sup>1</sup>					Agonism <sup>2</sup>					Antagonism <sup>3</sup>				
		+++	++	+	+/-	-	+++	++	+	+/-	-	###	##	#	#-	-
Amides	3				1						1					
Amines	2		1													
Amino Acids	1					P <sup>6</sup>					P					P
Azides	1					P					P					P
Carboxylic Acids	5		1	1	1	1		1	1							1
Esters	1				1				1							1
Flavonoids	9	2	3	1		1	1	1	3	2		2		1	1	3
Heterocyclic Compounds	23	3	4	2	1	1	1	1	4	2	3	2		2	1	4
Hydrocarbons (Cyclic)	6	3		1			1	1	1	1	1	2		1		2
Hydrocarbons (Halogenated)	5		2	1	1				5						1	3
Imidazoles	1					P					P					P
Indoles	1					P					P					P
Ketones	1					P					P					P
Lactones	1					P					P					P
Onium Compounds	1					P					P					P
Phenols	8		4	1				3	1					1		1
Phthalic Acids	3				2	1		1	1	1						2
Polycyclic Compounds	4					2					3		1			1
Pyrimidines	3									1	1				1	
Salts (Inorganic)	1					P					P					P
Steroids	22	5	3	3	1	2	4	3		4	7					5
Ureas	1										1					
<b>Totals<sup>7</sup></b>	<b>102</b>	<b>13</b>	<b>18</b>	<b>10</b>	<b>8</b>	<b>17</b>	<b>7</b>	<b>11</b>	<b>17</b>	<b>11</b>	<b>26</b>	<b>6</b>	<b>1</b>	<b>5</b>	<b>4</b>	<b>34</b>

<sup>1</sup> +++ Indicates that the substance was strongly active as measured by relative binding affinity (RBA) compared to estradiol (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 IC<sub>50</sub> value 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 (IC<sub>50</sub>) is the concentration of test substances that displaces 50% of the radiolabeled reference estrogen from the receptor.

<sup>2</sup>+++ Indicates that the substance was strongly active (the EC<sub>50</sub> value was <0.001 µM); ++ indicates that the substance was moderately active (EC<sub>50</sub> value was between 0.001 and 0.1 µM); + indicates that the substance was weakly active (EC<sub>50</sub> value was >0.1 µM), or a positive response was reported without an EC<sub>50</sub> value. The EC<sub>50</sub> is the effective concentration that causes half-maximal activation of the receptor.

<sup>3</sup> ### 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.

<sup>4</sup> Substances were assigned into one or more chemical classes using the U.S. National Library of Medicine's Medical Subject Headings (MeSH; Available: <http://www.nlm.nih.gov/mesh>), an internationally recognized standardized classification scheme.

<sup>5</sup> The number of substances indicated in the binding, agonism and antagonism columns may not add up to the number reflected in the distribution substances column because information on these attributes is not available for all substances on the ICCVAM recommended substances list.

<sup>6</sup> P = Substance is presumed to be negative.

<sup>7</sup> The total number is greater than the total number of proposed reference substances because some substances were assigned to more than one chemical class.

**Table 10 Revised ICCVAM Minimum List of 53 Reference Substances For Validation of *In Vitro* Estrogenic Receptor Binding and Transcriptional Activation Test Methods – Distribution of Substances by Chemical Class and Available Activity Data**

MeSH <sup>4</sup> Chemical Classes	Distribution of Substances <sup>5</sup>	Binding <sup>1</sup>					Agonism <sup>2</sup>					Antagonism <sup>3</sup>				
		+++	++	+	+/-	-	+++	++	+	+/-	-	###	##	#	#-	-
Amides	1				1											
Amines	1		1													
Azides	1					P <sup>6</sup>					P					P
Carboxylic Acids	5		1	1	1	1		1	1							1
Esters	1				1				1							1
Flavonoids	8	2	3	1		1	1	3	2		2		1	1	3	
Heterocyclic Compounds	15	2	4	2	1	1	1	1	3	2	3	2	1	1	4	
Hydrocarbons (Cyclic)	5	3	1				1	1	1	1	1	2	1		2	
Hydrocarbons (Halogenated)	5		2	1	1				5				1		3	
Phenols	8		4	2				3	2				1		1	
Phthalic Acids	3				2	1		1	1	1					2	
Polycyclic Compounds	3					2					2		1		1	
Pyrimidines	2					P					1				P	
Salts (Inorganic)	1					P					P				P	
Steroids	13	5	2	2	1	2	4	3		3	2				4	
<b>Totals<sup>7</sup></b>	<b>76</b>	<b>10</b>	<b>18</b>	<b>9</b>	<b>8</b>	<b>12</b>	<b>6</b>	<b>11</b>	<b>17</b>	<b>9</b>	<b>12</b>	<b>6</b>	<b>1</b>	<b>5</b>	<b>2</b>	<b>26</b>

<sup>1</sup>+++ Indicates that the substance was strongly active as measured by relative binding affinity (RBA) compared to estradiol (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 IC<sub>50</sub> value 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 (IC<sub>50</sub>) is the concentration of test substances that displaces 50% of the radiolabeled reference estrogen or androgen from the receptor.

<sup>2</sup>+++ Indicates that the substance was strongly active (EC<sub>50</sub> value was <0.001 μM); ++ indicates that the substance was moderately active (EC<sub>50</sub> value was between 0.001 and 0.1 μM); + indicates that the substance was weakly active (EC<sub>50</sub> value was >0.1 μM), or a positive response was reported without an EC<sub>50</sub> value. The EC<sub>50</sub> is the effective concentration that causes half-maximal activation of the receptor.

<sup>3</sup>### 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.

<sup>4</sup>Substances were assigned into one or more chemical classes using the U.S. National Library of Medicine's Medical Subject Headings (MeSH; Available: <http://www.nlm.nih.gov/mesh>), an internationally recognized standardized classification scheme.

<sup>5</sup>The number of substances indicated in the binding, agonism and antagonism columns may not add up to the number reflected in the distribution substances column because information on these attributes is not available for all substances on the ICCVAM recommended substances list.

<sup>6</sup>P = Substance is presumed to be negative.

<sup>7</sup>The total number is greater than the total number of proposed reference substances because some substances were assigned to more than one chemical class.

**Table 11 Revised ICCVAM List of 78 Reference Substances For Validation of *In Vitro* Androgenic Receptor Binding and Transcriptional Activation Test Methods – Distribution of Substances by Chemical Class and Available Activity Data**

MeSH <sup>4</sup> Chemical Classes	Distribution of Substances <sup>5</sup>	Binding <sup>1</sup>					Agonism <sup>2</sup>					Antagonism <sup>3</sup>				
		+++	++	+	+/-	-	+++	++	+	+/-	-	###	##	#	#-	-
Amides	3	1	2						2		1		2	1		
Amines	2					P <sup>6</sup>					P					P
Amino Acids	1					P					P					P
Azides	1					P					P					P
Carboxylic Acids	5					P					1					1
Esters	1					P					1					
Flavonoids	9										2			1		
Heterocyclic Compounds	23	1		1						2	5	1	1	1		2
Hydrocarbons (Cyclic)	6		1								2				2	
Hydrocarbons (Halogenated)	5		2	2				1			3			2	2	
Indoles	1					P					P					P
Imidazoles	1	1							1				1			
Ketones	1					P					P					P
Lactones	1	1							1				1			
Onium Compounds	1					P					P					P
Phenols	8									1	4	3		1	1	
Phthalic Acids	3					P					3					1
Polycyclic Compounds	4			1					1		1			2		
Pyrimidines	3					P					1					P
Salts (Inorganic)	1					P					P					P
Steroids	22	12	4	1		1	3	4	6		4	1	2		1	3
Ureas	1			1					1					1		
<b>Totals<sup>7</sup></b>	<b>103</b>	<b>16</b>	<b>9</b>	<b>6</b>	<b>0</b>	<b>13</b>	<b>3</b>	<b>5</b>	<b>11</b>	<b>4</b>	<b>36</b>	<b>5</b>	<b>7</b>	<b>9</b>	<b>6</b>	<b>16</b>

<sup>1</sup>+++ Indicates that the substance was strongly active as measured by relative binding affinity (RBA) compared to a reference androgen (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 IC<sub>50</sub> value 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 (IC<sub>50</sub>) is the concentration of test substances that displaces 50% of the radiolabeled reference androgen from the receptor.

<sup>2</sup>+++ Indicates that the substance was strongly active (EC<sub>50</sub> value was <0.001 µM); ++ indicates that the substance was moderately active (EC<sub>50</sub> value was between 0.001 and 0.1 µM); + indicates that the substance was weakly active (EC<sub>50</sub> value was >0.1 µM), or a positive response was reported without an EC<sub>50</sub> value. The EC<sub>50</sub> is the effective concentration that causes half-maximal activation of the receptor.

<sup>3</sup> ### 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.

<sup>4</sup> Substances were assigned into one or more chemical classes using the U.S. National Library of Medicine's Medical Subject Headings (MeSH; Available: <http://www.nlm.nih.gov/mesh>), an internationally recognized standardized classification scheme.

<sup>5</sup> The number of substances indicated in the binding, agonism and antagonism columns may not add up to the number reflected in the distribution substances column because information on these attributes is not available for all substances on the ICCVAM recommended substances list.

<sup>6</sup> P = Substance is presumed to be negative.

<sup>7</sup> The total number is greater than the total number of proposed reference substances because some substances were assigned to more than one chemical class.

**Table 12 Revised ICCVAM Minimum List of 44 Reference Substances For Validation of *In Vitro* Androgenic Receptor Binding and Transcriptional Activation Test Methods – Distribution of Substances by Chemical Class and Available Activity Data**

MeSH <sup>4</sup> Chemical Classes	Distribution of Substances <sup>5</sup>	Binding <sup>1</sup>					Agonism <sup>2</sup>					Antagonism <sup>3</sup>				
		+++	++	+	+/-	-	+++	++	+	+/-	-	###	##	#	#-	-
Amides	2	1	1						1				2			
Azides	1					P <sup>6</sup>					P					P
Carboxylic Acids	1					P					P					P
Esters	1										1					
Flavonoids	1					P					P					P
Heterocyclic Compounds	8	1		1					2	3	1	1				2
Hydrocarbons (Cyclic)	2		1								1			1		
Hydrocarbons (Halogenated)	5		2	2				1			3			2	2	
Imidazoles	1	1								1			1			
Lactones	1	1							1				1			
Phenols	3									1	2	1			1	
Phthalic Acids	2										2					
Polycyclic Compounds	3			1							1			2		
Pyrimidines	1										1					
Salts (Inorganic)	1					P					P					P
Steroids	18	11	4	1		1	3	4	6	0	3	1	2		1	3
Ureas	1			1					1					1		
<b>Totals<sup>7</sup></b>	<b>52</b>	<b>15</b>	<b>8</b>	<b>6</b>	<b>0</b>	<b>5</b>	<b>3</b>	<b>5</b>	<b>9</b>	<b>4</b>	<b>21</b>	<b>3</b>	<b>7</b>	<b>6</b>	<b>4</b>	<b>9</b>

<sup>1</sup> +++ Indicates that the substance was strongly active as measured by relative binding affinity (RBA) compared to a reference androgen (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 IC<sub>50</sub> value 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 (IC<sub>50</sub>) is the concentration of test substances that displaces 50% of the radiolabeled reference estrogen or androgen from the receptor.

<sup>2</sup> +++ Indicates that the substance was strongly active (EC<sub>50</sub> value was <0.001 µM); ++ indicates that the substance was moderately active (EC<sub>50</sub> value was between 0.001 and 0.1 µM); + indicates that the substance was weakly active (EC<sub>50</sub> value was >0.1 µM), or a positive response was reported without an EC<sub>50</sub> value. The EC<sub>50</sub> is the effective concentration that causes half-maximal activation of the receptor.

<sup>3</sup> ### 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.

<sup>4</sup> Substances were assigned into one or more chemical classes the U.S. National Library of Medicine's Medical Subject Headings (MeSH; Available: <http://www.nlm.nih.gov/mesh>), an internationally recognized standardized classification scheme.

<sup>5</sup> The number of substances indicated in the binding, agonism and antagonism columns may not add up to the number reflected in the distribution substances column because information on these attributes is not available for all substances on the ICCVAM recommended substances list.

<sup>6</sup> P = Substance is presumed to be negative.

<sup>7</sup> The total number is greater than the total number of proposed reference substances because some substances were assigned to more than one chemical class.



## 5.0 SUMMARY

ICCVAM has revised the 78 original substances recommended for use in future *in vitro* ER/AR binding and TA validation studies based on commercial availability and cost. This assessment indicated that three substances (anastrozole, CGS18320B, and fadrozole) are not commercially available and that the availability of ICI 182,780 is restricted. The assessment also indicated that six substances cost more than \$2000 for 500 mg, the expected minimum amount of substance required per laboratory to conduct an ED validation study. “Reasonable pricing” was defined as substances costing less than \$2000 per laboratory for a validation study. Based on this definition, actinomycin D, zearalenone, hydroxyflutamide, 4-hydroxytamoxifen, 12-*O*-tetradecanoylphorbol-13-acetate, and methyltrienolone did not meet reasonable pricing criteria. Actinomycin D has been retained despite its cost as it is the only RNA synthesis inhibitor on the current list of 78 reference substances and because it is listed as a minimum substance to be tested for both ER and AR validation studies.

Hydroxyflutamide has been retained as an ED reference substance because it was specifically recommended by the Panel, its AR activity is well documented in the scientific literature, and it is listed as a minimum substance to be tested for both ER and AR validation studies. 12-*O*-tetradecanoylphorbol-13-acetate will be retained because it is the only phorbol ester on the list of 78 recommended substances, it has known mitogenic activity that is not mediated via the ER pathway, and it is listed as a minimum substance to be tested for both ER and AR validation studies. 4-Hydroxytamoxifen has been retained because it is the active metabolite of tamoxifen and is therefore active in all cell based systems, it is well represented in the scientific literature, and it is listed as a minimum substance to be tested for both ER and AR validation studies.

Replacement substances were identified that have similar ER or AR binding or agonist TA activity profiles, or that are similarly concordant for antagonist TA activity across studies, and that are readily commercially available and are less expensive. Consideration for replacement substances was also based on whether the substance was on the original list of 122 ICCVAM ED candidate substances classified as “Substances Considered but not Included for Validation” or if the substance is being evaluated for ED activity by the EPA.

Replacement substances were identified based on the above criteria. Replacements were selected as follows:

- 4-OH androstenedione for anastrozole
- chrysin for CGS 18320B
- dicofol for fadrozole
- raloxifene for ICI 182,780
- 19-nortestosterone for methyltrienolone
- resveratrol for zearalenone

These replacements have similar ER or AR binding or agonist TA activity profiles, or are similarly concordant for antagonist TA activity across studies. Four substances, 4-OH androstenedione, dicofol, raloxifene, and 19-nortestosterone are on the original list of 122 ICCVAM candidate ED substances. Chrysin and dicofol are being evaluated for ED activity by the EPA. The ER data for resveratrol was obtained from a review of the current scientific literature.

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## **Appendix A**

### **Original ICCVAM Reference Substances for Validation of *In Vitro* Estrogen and Androgen Binding and TA Assays**

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### Appendix A Original ICCVAM Reference Substances for the Validation of *In Vitro* ER and AR Binding and TA Assays

Substance	CASRN <sup>a</sup>	MeSH <sup>b</sup> Chemical Class	Product Class	List of 78	Included on Minimum Lists		<i>In Vitro</i> Data (NICEATM) <sup>d</sup>			EPA	Proposed <i>In Vivo</i> Studies <sup>g,h,i,j</sup>	Comments
					ER Assays	AR Assays	Binding <sup>c</sup>	Transcriptional Activation				
								Agonism <sup>d</sup>	Antag <sup>e,f</sup>			
<b>Substances Proposed for Validation</b>												
Actinomycin D	50-76-0	Heterocyclic Compound, Polycyclic Compound	Laboratory Chemical, Pharmaceutical, Veterinary Agent	† <sup>k</sup>	ER	AR	1					Inhibits protein synthesis; recommended by the Expert Panel
Ammonium perchlorate	7790-98-9	Amine, Onium Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical	†	-	-				Y <sup>n</sup>	**	Thyroid disruptor; being considered for testing <i>in vivo</i> by EPA
<b>Anastrozole</b>	<b>120511-73-1</b>	<b>Nitrile</b>	<b>Pharmaceutical</b>	†	-	-		<b>AR-</b>		<b>Y</b>	<b>IM</b>	<b>Aromatase inhibitor; being tested <i>in vivo</i> by EPA</b>
4-Androstene dione	63-05-8	Steroid	Pharmaceutical	†	ER	AR	ER+/ AR+++	ER- /AR+++		Y		strong AR agonist; being tested <i>in vitro</i> by EPA
Apigenin	520-36-5	Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate	†	ER	-	ER+++	ER+++	ER#-	Y	**	strong ER agonist; being considered for testing <i>in vivo</i> by EPA
Apomorphine	58-00-4	Heterocyclic Compound	Pharmaceutical, Veterinary Agent	†	-	-				Y	IM	dopamine D1/D2 receptor agonist; being tested <i>in vivo</i> by EPA
Atrazine	1912-24-9	Heterocyclic Compound	Herbicide	†	ER	AR	ER+/AR+	ER-/AR-	ER-/AR-	Y	M-PA; **	Binds weakly to AR and ER; being tested <i>in vitro</i> and <i>in vivo</i> by EPA

Substance	CASRN <sup>a</sup>	MeSH <sup>b</sup> Chemical Class	Product Class	List of 78	Included on Minimum Lists		In Vitro Data (NICEATM) <sup>a</sup>			EPA	Proposed In Vivo Studies <sup>g,h,i,j</sup>	Comments
					ER Assays	AR Assays	Binding <sup>c</sup>	Transcriptional Activation				
								Agonism <sup>d</sup>	Antag <sup>e,f</sup>			
Bicalutamide	90357-06-5	Amide	Pharmaceutical	†	-	AR	AR+++	AR+	AR##			AR antagonist; recommended by the Expert Panel
Bisphenol A	80-05-7	Phenol	Chemical Intermediate, Flame Retardant, Fungicide	†	ER	AR	ER++	ER+/AR-	ER- /AR#-	Y	U; F-PA	weak ER agonist; being tested <i>in vitro</i> and <i>in vivo</i> by EPA and <i>in vivo</i> by OECD
Bisphenol B	77-40-7	Phenol	Chemical Intermediate, Flame Retardant, Fungicide	†	ER	-	ER++	ER++/AR-		Y		ER agonist; being tested <i>in vitro</i> by EPA
Butylbenzyl phthalate	85-68-7	Carboxylic Acid, Phthalic Acid	Chemical Intermediate, Plasticizer	†	ER	-	ER±	ER++/AR-	ER-/AR-	Y	**	ER agonist; being considered for testing <i>in vivo</i> by EPA
2-sec- Butylphenol	89-72-5	Phenol	Chemical Intermediate, Pesticide Intermediate, Plasticizer	†	ER	-	ER+			Y		binds weakly to ER; being tested <i>in vitro</i> by EPA
<b>CGS 18320B</b>	<b>112808-99-8</b>	<b>Heterocyclic Compound, Imidazole</b>	<b>Pharmaceutical</b>	†	-	-					<b>407</b>	<b>Aromatase inhibitor; being tested <i>in vivo</i> by OECD</b>
Clomiphene citrate	50-41-9	Amine, Carboxylic Acid, Heterocyclic Compound	Pharmaceutical	†	ER	-	ER++			Y		Binds to ER; being tested <i>in vitro</i> by EPA; recommended by the Expert Panel
Corticosterone	50-22-6	Steroid	Pharmaceutical	†	ER	AR	ER-/AR+	ER-/AR-		Y		binds weakly to AR; being tested <i>in vitro</i> by EPA

Substance	CASRN <sup>a</sup>	MeSH <sup>b</sup> Chemical Class	Product Class	List of 78	Included on Minimum Lists		In Vitro Data (NICEATM) <sup>a</sup>			EPA	Proposed In Vivo Studies <sup>g,h,i,j</sup>	Comments
					ER Assays	AR Assays	Binding <sup>c</sup>	Transcriptional Activation				
								Agonism <sup>d</sup>	Antag <sup>e,f</sup>			
Coumestrol	479-13-0	Heterocyclic Compound	Natural Product	†	ER	-	ER+++	ER++/AR-	ER-	Y	IM	ER agonist; being tested <i>in vitro</i> and <i>in vivo</i> by EPA
4-Cumylphenol	599-64-4	Phenol	Chemical Intermediate	†	ER	-		ER+/AR-		Y		weak ER agonist; being tested <i>in vitro</i> by EPA
Cycloheximide	66-81-9	Heterocyclic Compound	Fungicide, Pharmaceutical, Veterinary Agent	†	-	-						inhibits protein synthesis; recommended by the Expert Panel as a negative control
Cyproterone acetate	427-51-0	Steroid	Pharmaceutical	†	-	AR	AR+++	ER-/AR+	AR##	Y	IM	AR agonist and antagonist; being tested <i>in vitro</i> and <i>in vivo</i> by EPA
Daidzein	486-66-8	Flavonoid, Heterocyclic Compound	Natural Product	†	ER	-	ER++	ER+	ER-	Y		weak ER agonist; being tested <i>in vitro</i> by EPA
<i>p,p'</i> -DDE*	72-55-9	Hydrocarbon (Halogenated)	Pesticide Intermediate	†	ER	AR	ER±/ AR++	ER+/AR±	ER-/ AR#-	Y	H/407; M- PA; IM	weak AR agonist and antagonist; being tested <i>in vitro</i> and <i>in vivo</i> by EPA and <i>in vivo</i> by OECD
<i>o,p'</i> -DDT*	789-02-6	Hydrocarbon (Halogenated)	Pesticide	†	ER	AR	ER+++/ AR+	ER+/AR-	ER#/ AR#		U	weak ER agonist and antagonist; weak AR antagonist; being tested <i>in vivo</i> by OECD
Dexamethasone	50-02-2	Steroid	Pharmaceutical, Veterinary Agent	†	ER	AR	ER-/AR-	ER±/AR+		Y		weak ER and AR agonist; being tested <i>in vitro</i> by EPA

Substance	CASRN <sup>a</sup>	MeSH <sup>b</sup> Chemical Class	Product Class	List of 78	Included on Minimum Lists		In Vitro Data (NICEATM) <sup>a</sup>			EPA	Proposed In Vivo Studies <sup>g,h,i,j</sup>	Comments
					ER Assays	AR Assays	Binding <sup>c</sup>	Transcriptional Activation				
								Agonism <sup>d</sup>	Antag <sup>e,f</sup>			
Dibenzo[ <i>a,h</i> ] anthracene	53-70-3	Polycyclic Compound	Laboratory Chemical, Natural Product	†	ER	-	ER-	ER-/AR+	ER##			ER antagonist; included as it belongs to an under-represented class of substances
Di- <i>n</i> -butyl phthalate	84-74-2	Phthalic Acid	Pesticide Intermediate, Plasticizer	†	ER	AR	ER±	ER+/AR-	ER-	Y	U; M-PA; 1G	ER agonist; being tested <i>in vivo</i> by EPA and OECD
Diethylhexyl phthalate	117-81-7	Ester, Phthalic Acid	Cosmetic Ingredient, Industrial Chemical, Plasticizer	†	ER	AR	ER-	AR-		Y		being tested <i>in vitro</i> by EPA
Diethylstilbestrol	56-53-1	Hydrocarbon (Cyclic)	Pharmaceutical, Veterinary Agent	†	ER	AR	ER+++/ AR++	ER+++/ AR-	AR#	Y	**	ER agonist; being considered for testing <i>in vivo</i> by EPA
5 $\alpha$ -Dihydro testosterone***	521-18-6	Steroid	Pharmaceutical	†	ER	AR	ER+++/ AR+++	ER+/ AR+++		Y	H	weak ER agonist; strong AR agonist; being tested <i>in vitro</i> by EPA and <i>in vivo</i> by OECD
17 $\alpha$ -Estradiol	57-91-0	Steroid	Pharmaceutical, Veterinary Agent	†	ER	-	ER+++	ER++/AR-		Y		ER agonist; being tested <i>in vitro</i> by EPA
17 $\beta$ -Estradiol***	50-28-2	Steroid	Pharmaceutical, Veterinary Agent	†	ER	AR	ER+++/ AR++	ER+++/ AR++	AR##	Y	IM; **; FRS	strong ER agonist; AR agonist and antagonist; being tested <i>in vitro</i> and <i>in vivo</i> by EPA
Estrone	53-16-7	Steroid	Pharmaceutical, Veterinary Agent	†	ER	AR	ER+++/ AR++	ER+++/ AR++		Y		strong ER agonist; AR agonist; being tested <i>in vitro</i> by EPA

Substance	CASRN <sup>a</sup>	MeSH <sup>b</sup> Chemical Class	Product Class	List of 78	Included on Minimum Lists		In Vitro Data (NICEATM) <sup>a</sup>			EPA	Proposed In Vivo Studies <sup>g,h,i,j</sup>	Comments
					ER Assays	AR Assays	Binding <sup>c</sup>	Transcriptional Activation				
								Agonism <sup>d</sup>	Antag <sup>e,f</sup>			
17 $\alpha$ -Ethinyl estradiol	57-63-6	Steroid	Pharmaceutical, Veterinary Agent	†	ER	AR	ER+++/ AR++	ER+++/ AR-		Y	U/407; F- PA	strong ER agonist; being tested <i>in vitro</i> and <i>in vivo</i> by EPA and <i>in vivo</i> by OECD
Ethyl paraben	120-47-8	Carboxylic Acid, Phenol	Pharmaceutical, Preservative	†	ER	-				Y		being tested <i>in vitro</i> by EPA
<b>Fadrozole</b>	<b>102676-47-1</b>	<b>Imidazole, Nitrile</b>	<b>Pharmaceutical</b>	†	<b>ER</b>	<b>AR</b>				<b>Y</b>	<b>F-PA; IM; FRS</b>	<b>Aromatase inhibitor; being tested <i>in vivo</i> by EPA</b>
Fenarimol	60168-88-9	Heterocyclic Compound, Pyrimidine	Fungicide	†	-	-		ER+	ER#	Y	F-PA	Aromatase inhibitor; being tested <i>in vivo</i> by EPA
Finasteride	98319-26-7	Steroid	Pharmaceutical	†	-	AR		AR-	AR-	Y	H; M-PA; IM	5 $\alpha$ -reductase inhibitor; being tested <i>in vivo</i> by EPA and by OECD
Flavone	525-82-6	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical	†	ER	-	ER-	ER $\pm$	ER###	Y	M-PA; IM	ER antagonist; being tested <i>in vivo</i> by EPA
Fluoranthene	206-44-0	Polycyclic Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical Intermediate	†	ER	AR	ER-	ER-	ER- /AR#			AR antagonist; included as it belongs to an under-represented class of substances
Fluoxymestrone	76-43-7	Steroid	Pharmaceutical	†	-	AR	AR++	AR+	AR-			weak AR agonist; recommended by the Expert Panel
Flutamide	13311-84-7	Amide	Pharmaceutical, Veterinary Agent	†	-	-	AR++	ER-/AR-	AR#	Y	H/407; M- PA; IM; FRS	AR antagonist; being tested <i>in vivo</i> by EPA and by OECD

Substance	CASRN <sup>a</sup>	MeSH <sup>b</sup> Chemical Class	Product Class	List of 78	Included on Minimum Lists		In Vitro Data (NICEATM) <sup>a</sup>			EPA	Proposed In Vivo Studies <sup>g,h,i,j</sup>	Comments
					ER Assays	AR Assays	Binding <sup>c</sup>	Transcriptional Activation				
								Agonism <sup>d</sup>	Antag <sup>e,f</sup>			
Genistein	446-72-0	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical	†	ER	-	ER++	ER+	ER#		U/407	weak ER agonist and antagonist; being tested <i>in vivo</i> by OECD
Haloperidol	52-86-8	Ketone	Pharmaceutical, Veterinary Agent	†	-	-				Y	IM	dopamine D2 receptor antagonist; being tested <i>in vivo</i> by EPA
<i>meso</i> -Hexestrol	84-16-2	Steroid	Pharmaceutical, Veterinary Agent	†	ER	-	ER+++	ER+++		Y		strong ER agonist; being tested <i>in vitro</i> by EPA
Hydroxy flutamide	52806-53-8	Amide	Pharmaceutical	†	ER	AR	ER±/ AR++	AR+	AR##			AR agonist and antagonist; recommended by the Expert Panel
4-Hydroxy tamoxifen	68047-06-3	Hydrocarbon (Cyclic)	Pharmaceutical	†	ER	AR	ER+++	ER±/AR-	ER###			ER antagonist; recommended by the Expert Panel
<b>ICI 182,780</b>	<b>129453-61-8</b>	<b>Steroid</b>	<b>Pharmaceutical</b>	†	<b>ER</b>	-	<b>ER+++</b>	<b>ER-/AR-</b>	<b>ER###/ AR-</b>	<b>Y</b>	<b>IM</b>	<b>ER antagonist; being tested in vivo by EPA</b>
Kaempferol	520-18-3	Flavonoid, Heterocyclic Compound	Natural Product	†	ER	-	ER++	ER+	ER-	Y		weak ER agonist; being tested <i>in vitro</i> by EPA
Kepone	143-50-0	Hydrocarbon (Halogenated)	Pesticide	†	ER	AR	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	Heterocyclic Compound	Pharmaceutical	†	-	AR		AR±	AR-	Y	F and M- PA; IM	weak AR agonist; being tested <i>in vivo</i> by EPA



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					ER Assays	AR Assays	Binding <sup>c</sup>	Transcriptional Activation				
								Agonism <sup>d</sup>	Antag <sup>e,f</sup>			
Linuron	330-55-2	Urea	Herbicide	†	-	AR	AR+	ER-/AR+	AR#	Y	H; M-PA	weak AR agonist and antagonist; being tested <i>in vitro</i> and <i>in vivo</i> by EPA and <i>in vivo</i> by OECD
Medroxy progesterone acetate	71-58-9	Steroid	Pharmaceutical	†	-	AR	AR+++	AR+		Y		weak AR agonist; being tested <i>in vitro</i> by EPA
<i>p,p'</i> -Methoxychlor	72-43-5	Hydrocarbon (Halogenated)	Pesticide, Veterinary Agent	†	ER	AR	ER+/AR+	ER+/AR-	ER-/AR#	Y	U; F and M-PA; IM; **, 2G (avian)/FRS	weak ER agonist; AR antagonist; being tested <i>in vitro</i> and <i>in vivo</i> by EPA and <i>in vivo</i> by OECD
Methyl testosterone	58-18-4	Steroid	Pharmaceutical, Veterinary Agent	†	ER	AR	AR+++	ER++/AR++		Y	H/407; M-PA; **, FRS	ER and AR agonist; being tested <i>in vivo</i> by EPA and by OECD
<b>Methyl trienolone***</b>	<b>965-93-5</b>	<b>Steroid</b>	<b>Pharmaceutical</b>	†	-	<b>AR</b>	<b>AR+++</b>	<b>ER-/AR+</b>	<b>AR-</b>			<b>weak AR agonist</b>
Mifepristone	84371-65-3	Steroid	Pharmaceutical	†	-	AR	AR+++	ER-/AR++	AR###	Y	IM	AR agonist and antagonist; being tested <i>in vivo</i> by EPA
Morin	480-16-0	Flavonoid, Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate	†	ER	-	ER+			Y		binds weakly to ER; being tested <i>in vitro</i> by EPA
Nilutamide	63612-50-0	Heterocyclic Compound, Imidazole	Pharmaceutical	†	-	AR	AR+++	AR±	AR##			AR antagonist; recommended by the Expert Panel

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					ER Assays	AR Assays	Binding <sup>c</sup>	Transcriptional Activation				
								Agonism <sup>d</sup>	Antag <sup>e,f</sup>			
<i>p</i> -n-Nonylphenol	104-40-5	Phenol	Chemical Intermediate	†	ER	AR	ER++	ER++/AR±	ER#/ AR###	Y	U/407	ER agonist and antagonist; AR antagonist: being tested <i>in vitro</i> by EPA and <i>in vivo</i> by OECD
Norethynodrel	68-23-5	Steroid	Pharmaceutical	†	ER	-	ER++			Y		Binds to ER; being tested <i>in vitro</i> by EPA
<i>4-tert</i> - Octylphenol	140-66-9	Phenol	Chemical Intermediate, Pharmaceutical Intermediate	†	ER	AR	ER++	ER++/AR-		Y		ER agonist; being tested <i>in vitro</i> by EPA
Oxazepam	604-75-1	Heterocyclic Compound	Pharmaceutical, Veterinary Agent	†	-	-				Y	IM	enhances thyroid hormone excretion; being tested <i>in vivo</i> by EPA
Phenobarbital	57-30-7	Heterocyclic Compound, Pyrimidine	Pharmaceutical, Veterinary Agent	†	ER	AR		ER-/AR-		Y	F and M- PA; IM	enhances thyroid hormone excretion; being tested <i>in vivo</i> by EPA
Phenolphthalin	81-90-3	Carboxylic Acid, Phenol	Dye, Laboratory Chemical	†	ER	-	ER+			Y		binds weakly to ER; being tested <i>in vitro</i> by EPA
Pimozide	2062-78-4	Heterocyclic Compound	Pharmaceutical	†	-	-				Y	F and M- PA	Dopamine receptor antagonist; being tested <i>in vivo</i> by EPA
Procymidone	32809-16-8	Polycyclic Compound	Fungicide	†	-	AR	AR+	ER-/AR-	AR#	Y	H	AR antagonist; being tested <i>in vitro</i> by EPA and <i>in vivo</i> by OECD
Progesterone	57-83-0	Steroid	Pharmaceutical, Veterinary Agent	†	ER	AR	ER+/ AR+++	ER±/AR+	ER-/ AR#-	Y	IM	AR agonist; being tested <i>in vitro</i> and <i>in vivo</i> by EPA

Substance	CASRN <sup>a</sup>	MeSH <sup>b</sup> Chemical Class	Product Class	List of 78	Included on Minimum Lists		In Vitro Data (NICEATM) <sup>a</sup>			EPA	Proposed In Vivo Studies <sup>g,h,i,j</sup>	Comments
					ER Assays	AR Assays	Binding <sup>c</sup>	Transcriptional Activation				
								Agonism <sup>d</sup>	Antag <sup>e,f</sup>			
Propylthiouracil	51-52-5	Heterocyclic Compound, Pyrimidine	Pharmaceutical, Veterinary Agent	†	ER	-				Y	407; F and M-PA; **; 2G	inhibits T3/T4 synthesis; being tested <i>in vivo</i> by EPA and by OECD
Reserpine	50-55-5	Heterocyclic Compound, Indole	Pharmaceutical, Veterinary Agent	†	-	-				Y	IM	Depletes dopamine; being tested <i>in vivo</i> by EPA
Sodium azide	26628-22-8	Azide, Salt (Inorganic)	Chemical Intermediate, Fungicide, Herbicide	†	ER	AR						Cytotoxicant; recommended by the Expert Panel as a negative control
Spironolactone	52-01-7	Lactone, Steroid	Pharmaceutical	†	-	AR	AR+++	AR+	AR##	Y		AR agonist and antagonist; being tested <i>in vitro</i> by EPA
Tamoxifen	10540-29-1	Hydrocarbon (Cyclic)	Pharmaceutical	†	ER	-	ER+++	ER±/AR-	ER###			ER antagonist; Tamoxifen citrate is being tested <i>in vitro</i> by EPA
Testosterone	58-22-0	Steroid	Pharmaceutical, Veterinary Agent	†	ER	AR	ER±/ AR+++	ER±/ AR+++	AR-	Y	IM	strong AR agonist; being tested <i>in vitro</i> and <i>in vivo</i> by EPA
12-O- Tetradecanoyl phorbol-13- acetate	16561-29-8	Hydrocarbon (Cyclic)	Laboratory Chemical	†	ER	AR						activates ligand independent cell division; recommended by the Expert Panel
L-Thyroxine	51-48-9	Amino Acid	Pharmaceutical, Veterinary Agent	†	-	-					407	thyroid hormone; being tested <i>in vivo</i> by OECD
17β-Trenbolone	10161-33-8	Steroid	Pharmaceutical	†	-	AR	AR+++	ER-		Y	H	binds strongly to the AR; being tested <i>in vivo</i> by OECD

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					ER Assays	AR Assays	Binding <sup>c</sup>	Transcriptional Activation				
								Agonism <sup>d</sup>	Antag <sup>e,f</sup>			
2,4,5-Trichloro phenoxyacetic acid	93-76-5	Carboxylic Acid	Herbicide	†	ER	AR	ER-	ER+		Y		weak ER agonist; being tested <i>in vitro</i> by EPA
Vinclozolin	50471-44-8	Heterocyclic Compound	Fungicide	†	ER	AR	ER±/ AR++	ER-/AR-	AR###	Y	H; M-PA; IM; **; 1G/FRS	AR antagonist; being tested <i>in vitro</i> and <i>in vivo</i> by EPA and <i>in vivo</i> by OECD
<b>Zearalenone</b>	<b>17924-92-4</b>	<b>Lactone, Phenol</b>	<b>Chemical Intermediate, Veterinary Agent</b>	†	<b>ER</b>	<b>-</b>	<b>ER+++</b>	<b>ER++/AR-</b>	<b>ER#-</b>			<b>ER agonist; included as it belongs to an under-represented class of substances</b>
<b>Substances Considered but not Included for Validation</b>												
Arochlor 1254	11097-69-1	Hydrocarbon (Halogenated)	Chemical Intermediate, Pesticide Intermediate, Plasticizer		-	-	ER-	ER-		Y	**	does not bind to ER; omitted due to disposal concerns
Bendiocarb	22781-23-3	Carboxylic Acid	Pesticide		-	-		ER-	ER#			ER antagonist but no <i>in vivo</i> testing planned
Bisphenol C 2	14868-03-2	Phenol	Chemical Intermediate, Flame Retardant, Fungicide		-	-	ER+++					binds strongly to the ER but no <i>in vivo</i> testing planned
4- <i>tert</i> - Butylphenol	98-54-4	Phenol	Chemical Intermediate		-	-	ER±	ER+				weak ER agonist but no <i>in vivo</i> testing planned
Chlordane	57-74-9	Hydrocarbon (Halogenated)	Pesticide		-	-	ER-	ER+				weak ER agonist but no <i>in vivo</i> testing planned

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					ER Assays	AR Assays	Binding <sup>c</sup>	Transcriptional Activation				
								Agonism <sup>d</sup>	Antag <sup>e,f</sup>			
4-Chloro-4'- biphenylol	28034-99-3	Hydrocarbon (Cyclic)	-		-	-	ER+	ER+	ER-			weak ER agonist but no <i>in vivo</i> testing planned; concern regarding disposal
<i>Chrysin</i>	<i>480-40-0</i>	<i>Flavonoid, Heterocyclic Compound</i>	<i>Natural Product</i>		-	-				Y		<i>negative for ER and AR binding and no in vivo testing planned</i>
Cortisol	50-23-7	Steroid	Pharmaceutical, Veterinary Agent		-	-	ER-/AR-	ER-/AR++				AR agonist but no <i>in vivo</i> testing planned
Cyanoketone	4248-66-2	Steroid	Pharmaceutical		-	-	AR-					negative for ER and AR binding and no <i>in vivo</i> testing planned
<i>p,p'</i> -DDT*	50-29-3	Hydrocarbon (Halogenated)	Pesticide		-	-	ER+/AR+	ER+/AR-	AR##			weak ER agonist; AR antagonist; but no <i>in vivo</i> testing planned
<i>Dicofol</i>	<i>115-32-2</i>	<i>Hydrocarbon (Cyclic), Hydrocarbon (Halogenated)</i>	<i>Pesticide</i>		-	-		<i>ER+/AR-</i>	<i>ER-</i>	Y		<i>weak ER agonist but no in vivo testing planned</i>
Droloxifene	82413-20-5	Hydrocarbon (Cyclic)	Pharmaceutical		-	-	ER+++	ER±	ER###			ER antagonist but no <i>in vivo</i> testing planned
Equol	531-95-3	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical		-	-	ER++	ER+/AR-				ER agonist but no <i>in vivo</i> testing planned
Estriol	50-27-1	Steroid	Pharmaceutical, Veterinary Agent		-	-	ER+++/ AR-	ER++				ER agonist but no <i>in vivo</i> testing planned
Fenitrothion	122-14-5	Organo- phosphorus Compound	Pesticide		-	-		AR+	AR##			weak AR agonist but no <i>in vivo</i> testing planned

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					ER Assays	AR Assays	Binding <sup>c</sup>	Transcriptional Activation				
								Agonism <sup>d</sup>	Antag <sup>e,f</sup>			
Formononetin	485-72-3	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical		-	-	ER±	ER+	ER#			Weak ER agonist; ER antagonist but no <i>in vivo</i> testing planned
Genistin	529-59-9	Flavonoid, Heterocyclic Compound	Natural Product		-	-	ER±	ER±	ER-			Weak ER agonist but no <i>in vivo</i> testing planned
Heptachlor	76-44-8	Hydrocarbon (Halogenated)	Pesticide, Veterinary Agent		-	-	ER-	ER-				Does not bind to ER and no <i>in vivo</i> testing planned
<i>4-Hydroxy androstenedione</i>	<i>566-48-3</i>	<i>Steroid</i>	<i>Pharmaceutical</i>		-	-	<i>AR++</i>			<i>Y</i>		<i>Binds to AR but no in vivo testing planned</i>
17 $\alpha$ -Hydroxy progesterone	68-96-2	Steroid	Pharmaceutical, Veterinary Agent		-	-	AR++	ER-				Binds to AR but no <i>in vivo</i> testing planned
Hydroxy toremifene	110503-62-3	Hydrocarbon (Cyclic)	Pharmaceutical		-	-		ER±	ER###			ER antagonist but no <i>in vivo</i> testing planned
ICI 164,384	98007-99-9	Steroid	Pharmaceutical		-	-	ER+++	ER±	ER###			ER antagonist but no <i>in vivo</i> testing planned
Kaempferide	491-54-3	Flavonoid, Heterocyclic Compound	Natural Product		-	-		ER±	ER###			ER antagonist but no <i>in vivo</i> testing planned
Letrozole	112809-51-5	Nitrile	Pharmaceutical		-	-				<i>Y</i>	<i>F-PA (?)</i>	Aromatase inhibitor; questionable whether Letrozole will be tested <i>in vivo</i>
Levonorgestrel	797-63-7	Steroid	Pharmaceutical		-	-	AR+++	ER±/ AR+++				Weak ER agonist; strong AR agonist; but no <i>in vivo</i> testing planned

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					ER Assays	AR Assays	Binding <sup>c</sup>	Transcriptional Activation				
								Agonism <sup>d</sup>	Antag <sup>e,f</sup>			
Lindane	58-89-9	Hydrocarbon (Halogenated)	Pesticide, Veterinary Agent		-	-	ER±	ER+/AR-	AR-			Weak ER agonist but no <i>in vivo</i> testing planned
Melengestrol acetate	2919-66-6	Steroid	Pharmaceutical		-	-	AR++	ER+				Weak ER agonist but no <i>in vivo</i> testing planned
Mestranol	72-33-3	Steroid	Pharmaceutical		-	-	ER++	ER+				Weak ER agonist but no <i>in vivo</i> testing planned
Methyl parathion	298-00-0	Organo- phosphorus Compound	Pesticide		-	-		ER+		Y	2G (avian)	Being tested <i>in vivo</i> by EPA, but not considered as it is highly toxic
Mirex	2385-85-5	Hydrocarbon (Halogenated)	Flame Retardant, Pesticide		-	-	ER-	ER-/AR-	AR-			Does not bind to ER or AR and no <i>in vivo</i> testing planned
Nafoxidine	1845-11-0	Heterocyclic Compound	Pharmaceutical		-	-	ER++	ER±/AR-				Binds to ER but no <i>in vivo</i> testing planned
Naringenin	480-41-1	Flavonoid, Heterocyclic Compound	Natural Product		-	-	ER+	ER+	ER#-			Weak ER agonist but no <i>in vivo</i> testing planned
<i>19-Nor testosterone</i>	<i>434-22-0</i>	<i>Steroid</i>	<i>Pharmaceutical, Veterinary Agent</i>		-	-	<i>ER++/ AR+++</i>	<i>ER±/ AR+++</i>				<i>weak ER agonist; AR agonist; but no in vivo testing planned</i>
4-Octylphenol	1806-26-4	Phenol	Chemical Intermediate, Plasticizer		-	-	ER+	ER+	ER#			Weak ER agonist; ER antagonist but no <i>in vivo</i> testing planned
Phloretin	60-82-2	Ketone, Phenol	Natural Product		-	-	ER++	ER+	ER#-			weak ER agonist but no <i>in vivo</i> testing planned

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					ER Assays	AR Assays	Binding <sup>c</sup>	Transcriptional Activation				
								Agonism <sup>d</sup>	Antag <sup>e,f</sup>			
Pregnenolone	145-13-1	Steroid	Pharmaceutical		-	-	AR±					binds weakly to AR but no <i>in vivo</i> testing planned
<i>Raloxifene</i> <sup>m</sup>	84449-90-1	<i>Hydrocarbon (Cyclic)</i>	<i>Pharmaceutical</i>		-	-	<i>ER+++</i>	<i>ER-</i>	<i>ER###</i>	Y		<i>ER antagonist but no in vivo testing planned</i>
<i>Resveratrol</i>	501-36-0	<i>Hydrocarbon (Cyclic)</i>	<i>Natural Product</i>		-	-	<i>ER+</i>	<i>ER++</i>	<i>ER#/ AR#</i>	N		<i>Selected as a replacement substances based on review of the scientific literature</i>
Simazine	122-34-9	Heterocyclic Compound	Herbicide		-	-	ER-	ER±	ER-			weak ER agonist and no <i>in vivo</i> testing planned
β-Sitosterol	5779-62-4	Steroid	Natural Product, Pharmaceutical		-	-	ER-	ER±/AR-				weak ER agonist and no <i>in vivo</i> testing planned
Tamoxifen citrate	54965-24-1	Hydrocarbon (Cyclic)	Pharmaceutical		-	-	ER+++	ER-	ER#	Y		ER antagonist; being tested <i>in vitro</i> by EPA but no <i>in vivo</i> testing planned
Testosterone propionate	57-85-2	Steroid	Pharmaceutical		-	-					H	no binding or TA data; being tested by OECD <i>in vivo</i>



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					ER Assays	AR Assays	Binding <sup>c</sup>	Transcriptional Activation				
								Agonism <sup>d</sup>	Antag <sup>e,f</sup>			
2,3,7,8,- Tetrachloro dibenzo- <i>p</i> -dioxin	1746-01-6	Dioxin, Heterocyclic Compound	Laboratory Chemical		-	-	ER-	ER++/AR-	ER###/ AR#	Y	**	ER agonist and antagonist; not considered due to extreme toxicity
2',4',6',- Trichloro-4- biphenylol	14962-28-8	Hydrocarbon (Cyclic), Hydrocarbon (Halogenated)	Laboratory Chemical		-	-	ER+++	ER+	ER#-			weak ER agonist; no <i>in vivo</i> testing planned; concern regarding disposal
$\alpha$ -Zearalanol	26538-44-3	Lactone, Phenol	Natural Product		-	-	ER+++	ER++/AR-	ER##			ER agonist and antagonist; no <i>in vivo</i> testing planned
$\beta$ -Zearalanol	71030-11-0	Lactone, Phenol	Natural Product		-	-	ER+++	ER+				ER agonist; no <i>in vivo</i> testing planned

Substances listed in bolded text were originally included on the ICCVAM List of Reference Substances ER/AR Binding and TA Activation Assays, but have been replaced because of lack of commercial availability or excessive cost.

Substances that are both bolded and italicized are replacements for the substances that were not commercially available or had excessive cost.

EPA = United States Environmental Protection Agency

OECD = Organisation for Economic Co-operation and Development

\**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, *in utero* through lactation.

\*\*\* 17 $\beta$ -Estradiol is the recommended positive control substance for the ER binding and ER TA assays; for AR binding, 5 $\alpha$  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 5 $\alpha$  Dihydrotestosterone or Methyltrienolone is recommended as the positive control.

<sup>a</sup> CASRN = Chemical Abstracts Service Registry Number

<sup>b</sup> MeSH = Medical Subject Headings, information on chemical class criteria can be obtained at [www.nlm.nih.gov/MeSH](http://www.nlm.nih.gov/MeSH)

<sup>c</sup> +++ Indicates that the substance was strongly 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 IC<sub>50</sub> value was not obtained and thus an RBA value could not be determined;  $\pm$  indicates an equivocal response (i.e., in different studies, the substance was reported as positive and negative). The inhibitory concentration 50 (IC<sub>50</sub>) is the concentration of test substances that displaces 50% of the radiolabeled reference estrogen or androgen from the receptor.

<sup>d</sup> +++ Indicates that the substance was strongly active (EC<sub>50</sub> value was <0.001 µM); ++ indicates that the substance was moderately active (EC<sub>50</sub> value was between 0.001 and 0.1 µM); + indicates that the substance was weakly active (EC<sub>50</sub> value was >0.1 µM), or a positive response was reported without an EC<sub>50</sub> value. The EC<sub>50</sub> is the effective concentration that causes half-maximal activation of the receptor.

<sup>c</sup> Antag = Antagonist

<sup>f</sup> ### 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.

<sup>g</sup> 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 assesses the effect on all organs including the reproductive organs, of varying concentrations of the test substance administered daily to seven 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.

<sup>h</sup> 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. Histopathological 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).

<sup>i</sup> The *in utero* through lactation assay (Assesses post-natal development of neonates after *in utero* and lactational exposure) has been recommended, but the EPA has not made a decision for its further development or for validation.

<sup>j</sup> FRS = Fish Reproductive Screen; 1G = one generation; 2G = two generation.

<sup>k</sup> † Indicates that a substance was included in the ICCVAM list of 78 Reference Substances for the Validation of *In Vitro* and *In Vivo* ER and AR Binding and TA Assays.

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

<sup>m</sup> Raloxifene may act as an agonist in some *in vitro* systems.

<sup>n</sup> Y indicates that this substance is being evaluated for ED activity by the EPA.

## Appendix B

### Revised ICCVAM List of Reference Substances for Validation of *In Vitro* Binding and TA Assays

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## **Appendix B-1**

### **Revised ICCVAM List of Reference Substances for Validation of *In Vitro* ER Binding Assays Sorted by ER Binding Activity and Substance Name**

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**Appendix B-1 Revised ICCVAM List of Reference Substances for Validation of *In Vitro* ER Binding Assays Sorted by ER Binding Activity and Substance Name**

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Binding Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log Kow <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Apigenin	520-36-5	270.2	N <sup>j</sup>	+++	183 mg/L @ 25° C	27 mg/ml @ 25° C	-. <sup>h</sup>	\$790	Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate
Coumestrol	479-13-0	268.2	Y <sup>k</sup>	+++	practically insoluble	-	-	\$1,550	Heterocyclic Compound	Natural Product
Diethylstilbestrol	56-53-1	268.4	Y	+++	12 mg/L @ 25° C	-	5.07	\$47	Hydrocarbon (Cyclic)	Pharmaceutical, Veterinary Agent
17a-Estradiol	57-91-0	272.4	Y	+++	3.9 mg/L	-	3.94	\$230	Steroid	Pharmaceutical, Veterinary Agent
17a-Ethinyl estradiol	57-63-6	296.4	Y	+++	insoluble	-	3.67	\$35	Steroid	Pharmaceutical, Veterinary Agent
17β-Estradiol	50-28-2	272.4	Y	+++	3.60 mg/L @ 27° C	soluble	4.01	\$151	Steroid	Pharmaceutical, Veterinary Agent
Estrone	53-16-7	270.4	Y	+++	0.003 g/100 mL @ 25° C	-	3.13	\$14	Steroid	Pharmaceutical, Veterinary Agent
<i>meso</i> -Hexestrol	84-16-2	270.4	Y	+++	-	-	-	\$35	Steroid	Pharmaceutical, Veterinary Agent
4-Hydroxytamoxifen	68047-06-3	387.5	N	+++	practically insoluble	soluble	-	\$6,090	Hydrocarbon (Cyclic)	Pharmaceutical
Raloxifene <sup>i</sup>	82640-04-8	510.1	N	+++	insoluble	28 mg/ml	-	\$235	Hydrocarbon (Cyclic)	Pharmaceutical
Tamoxifen	10540-29-1	371.5	Y	+++	practically insoluble	soluble	-	\$125	Hydrocarbon (Cyclic)	Pharmaceutical

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Binding Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log Kow <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Bisphenol A	80-05-7	228.3	Y	++	120 mg/L @ 25° C	-	3.32	\$12	Phenol	Chemical Intermediate, Flame Retardant, Fungicide
Bisphenol B	77-40-7	242.3	Y	++	1 g in 50 mL	-	2.30	\$110	Phenol	Chemical Intermediate, Flame Retardant, Fungicide
Clomiphene citrate	50-41-9	598.1	Y	++	slightly soluble	-	-	\$45	Amine, Carboxylic Acid, Heterocyclic Compound	Pharmaceutical
Daidzein	486-66-8	254.2	Y	++	practically insoluble	10 mg/ml	-	\$735	Flavonoid, Heterocyclic Compound	Natural Product
<i>o,p'</i> -DDT*	789-02-6	354.5	Y	++	0.085 mg/L @ 25° C	-	6.79	\$714	Hydrocarbon (Halogenated)	Pesticide
5a-Dihydrotestosterone	521-18-6	290.4	Y	++	practically insoluble	-	3.55	\$27	Steroid	Pharmaceutical
Genistein	446-72-0	270.2	Y	++	insoluble	50 mg/ml @ 25° C	2.84	\$943	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
Kaempferol	520-18-3	286.2	Y	++	slightly soluble	-	1.96	\$390	Flavonoid, Heterocyclic Compound	Natural Product
Kepone	143-50-0	490.6	Y	++	2.70 mg/L @ 25° C	-	5.41	\$123	Hydrocarbon (Halogenated)	Pesticide
<i>p</i> -n -Nonylphenol	104-40-5	220.4	Y	++	-	-	5.76	\$192	Phenol	Chemical Intermediate
Norethynodrel	68-23-5	298.4	Y	++	practically insoluble	-	3.51	\$78	Steroid	Pharmaceutical
19-Nortestosterone	434-22-0	274.4	N	++	-	-	-	\$90	Steroid	Pharmaceutical, Veterinary Agent



Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Binding Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log Kow <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
4- <i>tert</i> -Octylphenol	140-66-9	206.3	Y	++	-	-	-	\$28	Phenol	Chemical Intermediate, Pharmaceutical Intermediate
<i>p,p'</i> -DDE*	72-55-9	318.0	Y	+-	0.04 mg/L 25 °C	-	6.51	\$94	Hydrocarbon (Halogenated)	Pesticide Intermediate
Hydroxyflutamide	52806-53-8	292.2	N	+-	27.5 mg/L @ 25 °C	-	2.7	\$2,941	Amide	Pharmaceutical
Butylbenzyl phthalate	85-68-7	312.4	Y	+-	2.69 mg/L @ 25° C	-	4.91	\$79	Carboxylic Acid, Phthalic Acid	Chemical Intermediate, Plasticizer
Di - <i>n</i> -butyl phthalate	84-74-2	278.3	Y	+-	-	-	-	\$34	Ester, Phthalic Acid	Cosmetic Ingredient, Industrial Chemical, Plasticizer
Testosterone	58-22-0	288.4	N	+-	practically insoluble	soluble	-	\$26	Steroid	Pharmaceutical, Veterinary Agent
Vinclozolin	50471-44-8	286.1	Y	+-	1000 mg/L @ 20 °C	-	3.10	\$41	Heterocyclic Compound	Fungicide
4-Androstenedione	63-05-8	286.4	N	+	57.8 mg/mL 25 °C	-	2.75	\$53	Steroid	Pharmaceutical
Atrazine	1912-24-9	215.7	Y	+	34.7 mg/L @ 26° C	183 g/kg @ 25° C	2.61	\$68	Heterocyclic Compound	Herbicide
2- <i>sec</i> -Butylphenol	89-72-5	150.2	N	+	insoluble	-	3.27	\$16	Phenol	Chemical Intermediate, Pesticide Intermediate, Plasticizer
<i>p,p'</i> - Methoxychlor	72-43-5	345.7	Y	+	30.5 mg/L @ 25 °C	-	5.08	\$17	Hydrocarbon (Halogenated)	Pesticide, Veterinary Agent
Morin	480-16-0	302.2	Y	+	250 mg/L @ 25 °C	-	1.54	\$14	Flavonoid, Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Binding Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log Kow <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Phenolphthalin	81-90-3	320.3	Y	+	175 mg/L @ 20 °C	-	3.95	\$26	Carboxylic Acid, Phenol	Dye, Laboratory Chemical
Progesterone	57-83-0	314.5	Y	+	8.8 mg/L @ 25 °C	-	3.87	\$25	Steroid	Pharmaceutical, Veterinary Agent
Resveratrol	501-36-0	228.2	N	+	-	-	3.08	\$226	Hydrocarbon (Cyclic)	Natural Product
Corticosterone	50-22-6	346.5	Y	-	199 mg/L @ 25 °C	-	-	\$47	Steroid	Pharmaceutical
Dexamethasone	50-02-2	392.5	Y	-	10 mg/100mL @ 25 °C	-	-	\$352	Steroid	Pharmaceutical, Veterinary Agent
Dibenzo[ <i>a,h</i> ]anthracene	53-70-3	278.4	Y	-	practically insoluble	-	6.5	\$352	Polycyclic Compound	Laboratory Chemical, Natural Product
Flavone	525-82-6	222.2	N	-	0.1 mg/L @ 25 °C	-	5.08	\$48	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
Fluoranthene	206-44-0	202.3	Y	-	0.2 mg/L	soluble	5.16	\$21	Polycyclic Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical Intermediate
Diethylhexyl phthalate	117-81-7	330.2	Y	-	0.285 mg/L @ 24 °C	-	7.6	\$26	Phthalic Acid	Pesticide Intermediate, Plasticizer
2,4,5-Trichlorophenoxyacetic acid	93-76-5	255.5	Y	-	278 mg/L	-	3.31	\$48	Carboxylic Acid	Herbicide
Actinomycin D	50-76-0	1255.4	Y	n.d. <sup>1</sup>	1 g/L at 37 °C	10 mg/ml	3.21 @ pH of 7.4	\$2,285	Heterocyclic Compound, Polycyclic Compound	Laboratory Chemical, Pharmaceutical, Veterinary Agent

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Binding Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log Kow <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Ammonium perchlorate	7790-98-9	117.5	N	n.d.	200 g/L at 25 °C	-	-	\$55	Amine, Onium Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical
4-Hydroxy androstenedione	566-48-3	302.4	Y	n.d.	-	-	-	\$107	Steroid	Pharmaceutical
Apomorphine	58-00-4	267.3	Y	n.d.	1.660 g/mL	-	2.3	\$428	Heterocyclic Compound	Pharmaceutical, Veterinary Agent
Bicalutamide	90357-06-5	430.4	Y	n.d.	5 mg/L	-	-	\$436	Amide	Pharmaceutical
<i>Chrysin</i>	<i>480-40-0</i>	<i>254.24</i>	<i>Y</i>	<i>n.d.</i>	<i>84 mg/L @ 25 °C</i>	-	<i>3.52</i>	<i>\$60</i>	<i>Flavonoid, Heterocyclic Compound</i>	<i>Natural Product</i>
<b>4-Cumylphenol</b>	<b>599-64-4</b>	<b>212.3</b>	<b>N</b>	<b>n.d.</b>	<b>insoluble</b>	-	-	<b>\$24</b>	<b>Phenol</b>	<b>Chemical Intermediate</b>
Cycloheximide	66-81-9	281.4	N	n.d.	0.00021 mg/L @ 25 °C	-	0.55	\$45	Heterocyclic Compound	Fungicide, Pharmaceutical, Veterinary Agent
Cyproterone acetate	427-51-0	416.9	Y	n.d.	-	-	-	\$268	Steroid	Pharmaceutical
<i>Dicofol</i>	<i>115-32-2</i>	<i>370.489</i>	<i>Y</i>	<i>n.d.</i>	<i>1.2 mg/L @ 24°C</i>	-	<i>4.28</i>	<i>\$88</i>	<i>Hydrocarbon (Cyclic), Hydrocarbon (Halogenated)</i>	<i>Pesticide</i>
Fenarimol	60168-88-9	331.2	Y	n.d.	14 mg/L @ 25 °C	-	3.6	\$375	Heterocyclic Compound, Pyrimidine	Fungicide
Finasteride	98319-26-7	372.5	Y	n.d.	-	-	-	\$377	Steroid	Pharmaceutical
Fluoxymestrone	76-43-7	336.4	Y	n.d.	practically insoluble	-	2.38	\$131	Steroid	Pharmaceutical
Flutamide	13311-84-7	276.2	Y	n.d.	9.45 mg/L @ 25 °C	soluble	3.35	\$22	Amide	Pharmaceutical, Veterinary Agent
Haloperidol	52-86-8	375.9	Y	n.d.	1.4 mg/L @ 25 °C	soluble	4.3	\$54	Ketone	Pharmaceutical, Veterinary Agent
Ketoconazole	65277-42-1	531.4	Y	n.d.	0.087 mg/L @ 25 °C	-	4.35	\$380	Heterocyclic Compound	Pharmaceutical
Linuron	330-55-2	249.1	Y	n.d.	75 mg/L @ 25 °C	-	3.2	\$124	Urea	Herbicide

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Binding Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log Kow <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Medroxyprogesterone acetate	71-58-9	386.5	Y	n.d.	practically insoluble	-	-	\$105	Steroid	Pharmaceutical
Mifepristone (Mifeprex)	84371-65-3	429.6	Y	n.d.	insoluble	-	-	\$262	Steroid	Pharmaceutical
Nilutamide	63612-50-0	317.2	Y	n.d.	insoluble	-	-	\$45	Heterocyclic Compound, Imidazole	Pharmaceutical
Oxazepam	604-75-1	286.7	Y	n.d.	179 mg/L	-	2.24	\$463	Heterocyclic Compound	Pharmaceutical, Veterinary Agent
<b>Ethyl paraben</b>	<b>120-47-8</b>	<b>166.2</b>	<b>Y</b>	<b>n.d.</b>	<b>885 mg/L @ 25 °C</b>	-	<b>2.47</b>	<b>\$8</b>	<b>Carboxylic Acid, Phenol</b>	<b>Pharmaceutical, Preservative</b>
<b>Phenobarbital</b>	<b>50-06-6</b>	<b>232.2</b>	<b>Y</b>	<b>n.d.</b>	<b>1300 mg/L @ 25 °C</b>	-	<b>1.47</b>	<b>\$56</b>	<b>Heterocyclic Compound, Pyrimidine</b>	<b>Pharmaceutical, Veterinary Agent</b>
Pimozide	2062-78-4	461.6	Y	n.d.	insoluble	18 mg/mL	-	\$45	Heterocyclic Compound	Pharmaceutical
Procymidone	32809-16-8	284.1	Y	n.d.	4.5 mg/L @ 25 °C	-	-	\$110	Polycyclic Compound	Fungicide
<b>Propylthiouracil</b>	<b>51-52-5</b>	<b>170.2</b>	<b>Y</b>	<b>n.d.</b>	<b>1204 mg/L @ 25 °C</b>	-	-	<b>\$28</b>	<b>Heterocyclic Compound, Pyrimidine</b>	<b>Pharmaceutical, Veterinary Agent</b>
Reserpine	50-55-5	608.7	Y	n.d.	73 mg/L @ 30 °C	-	-	\$76	Heterocyclic Compound, Indole	Pharmaceutical, Veterinary Agent
<b>Sodium azide</b>	<b>26628-22-8</b>	<b>65.0</b>	<b>Y</b>	<b>n.d.</b>	<b>41 g/100 mL @ 15 °C</b>	-	-	<b>\$17</b>	<b>Azide, Salt (inorganic)</b>	<b>Chemical Intermediate, Fungicide, Herbicide</b>
Spironolactone	52-01-7	416.6	Y	n.d.	22 mg/L @ 25 °C	soluble	2.78	\$38	Lactone, Steroid	Pharmaceutical
<b>Methyl testosterone</b>	<b>58-18-4</b>	<b>302.5</b>	<b>Y</b>	<b>n.d.</b>	-	-	<b>3.32</b>	<b>\$26</b>	<b>Steroid</b>	<b>Pharmaceutical, Veterinary Agent</b>
<b>12-O-Tetradecanoylphorbol-13-acetate</b>	<b>16561-29-8</b>	<b>616.8</b>	<b>N</b>	<b>n.d.</b>	-	<b>soluble</b>	-	<b>\$11,200</b>	<b>Hydrocarbon (Cyclic)</b>	<b>Laboratory Chemical</b>
L-Thyroxine	51-48-9	776.9	Y	n.d.	slightly soluble	-	-	\$65	Amino Acid	Pharmaceutical, Veterinary Agent
17b-Trenbolone	10161-33-8	270.4	Y	n.d.	-	-	-	\$130	Steroid	Pharmaceutical

Substances listed in bolded text are included on the ICCVAM Minimum List of Substances for Validation of ER binding and TA Assays.

\**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

<sup>a</sup> CASRN = Chemical Abstracts Service Registry Number

<sup>b</sup> MW = Molecular Weight

<sup>c</sup> +++ Indicates that the substance was strongly 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 IC<sub>50</sub> value 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 (IC<sub>50</sub>) is the concentration of test substances that displaces 50% of the radiolabeled reference estrogen or androgen from the receptor.

<sup>d</sup> Information on Solubility in Water, Solubility in DMSO, and log K<sub>ow</sub>, were obtained from the National Library of Medicine's ChemIDplus <http://chem.sis.nlm.nih.gov/chemidplus/>, and from manufacturer Materials Safety Data Sheets (MSDS).

<sup>e</sup> 500 mg is the expected minimum amount of substance required per laboratory to conduct an endocrine disruptor (ED) validation study.

<sup>f</sup> Pricing information was obtained from vendors during October of 2005 and reflects the cost of 500 mg of substance, or the minimum amount sold.

<sup>g</sup> MeSH = Medical Subject Headings, information on chemical class criteria can be obtained at [www.nlm.nih.gov/MeSH](http://www.nlm.nih.gov/MeSH)

<sup>h</sup> A “-“ in the fields for Solubility in Water, Solubility in DMSO or low K<sub>ow</sub> indicates that there is no data for this field.

<sup>i</sup> Raloxifene may act as an agonist in some *in vitro* systems.

<sup>j</sup> N indicates that this substance is not included on the EPA reference chemical list.

<sup>k</sup> Y indicates that this substance is included on the EPA reference chemical list.

<sup>l</sup> n.d. indicates that no relevant data were identified.

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## **Appendix B-2**

### **Revised ICCVAM List of Reference Substances for Validation of *In Vitro* ER TA Agonist Assays Sorted by ER Agonist Activity and Substance Name**

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**Appendix B-2 Revised ICCVAM List of Reference Substances for Validation of *In Vitro* ER TA Agonist Assays Sorted by ER TA Agonist Activity and Substance Name**

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Agonist Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Apigenin	520-36-5	270.2	N <sup>j</sup>	+++	183 mg/L @ 25° C	27 mg/ml @ 25° C	- <sup>h</sup>	\$790	Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate
Diethylstilbestrol	56-53-1	268.4	Y <sup>k</sup>	+++	12 mg/L @ 25° C	-	5.07	\$47	Hydrocarbon (Cyclic)	Pharmaceutical, Veterinary Agent
17a-Ethinyl estradiol	57-63-6	296.4	Y	+++	insoluble	-	3.67	\$35	Steroid	Pharmaceutical, Veterinary Agent
17β-Estradiol	50-28-2	272.4	Y	+++	3.60 mg/L @ 27° C	soluble	4.01	\$151	Steroid	Pharmaceutical, Veterinary Agent
Estrone	53-16-7	270.4	Y	+++	0.003 g/100 mL @ 25° C	-	3.13	\$14	Steroid	Pharmaceutical, Veterinary Agent
<i>meso</i> -Hexestrol	84-16-2	270.4	Y	+++	-	-	-	\$35	Steroid	Pharmaceutical, Veterinary Agent
Bisphenol B	77-40-7	242.3	Y	++	1 g in 50 mL	-	2.30	\$110	Phenol	Chemical Intermediate, Flame Retardant, Fungicide
Coumestrol	479-13-0	268.2	Y	++	practically insoluble	-	-	\$1,550	Heterocyclic Compound	Natural Product
5a-Dihydrotestosterone	521-18-6	290.4	Y	++	practically insoluble	-	3.55	\$27	Steroid	Pharmaceutical

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Agonist Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
17 $\alpha$ -Estradiol	57-91-0	272.4	Y	++	3.9 mg/L	-	3.94	\$230	Steroid	Pharmaceutical, Veterinary Agent
<i>p</i> -n -Nonylphenol	104-40-5	220.4	Y	++	-	-	5.76	\$192	Phenol	Chemical Intermediate
4- <i>tert</i> -Octylphenol	140-66-9	206.3	Y	++	-	-	-	\$28	Phenol	Chemical Intermediate, Pharmaceutical Intermediate
Butylbenzyl phthalate	85-68-7	312.4	Y	++	2.69 mg/L @ 25° C	-	4.91	\$79	Carboxylic Acid, Phthalic Acid	Chemical Intermediate, Plasticizer
Resveratrol	501-36-0	228.2	N	++	-	-	3.08	\$226	Hydrocarbon (Cyclic)	Natural Product
Methyl testosterone	58-18-4	302.5	Y	++	-	-	3.32	\$26	Steroid	Pharmaceutical, Veterinary Agent
Dexamethasone	50-02-2	392.5	Y	+-	10 mg/100mL @ 25° C	-	-	\$352	Steroid	Pharmaceutical, Veterinary Agent
Flavone	525-82-6	222.2	N	+-	0.1 mg/L @ 25° C	-	5.08	\$48	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
4-Hydroxytamoxifen	68047-06-3	387.5	N	+-	practically insoluble	soluble	-	\$6,090	Hydrocarbon (Cyclic)	Pharmaceutical
19-Nortestosterone	434-22-0	274.4	N	+-	-	-	-	\$90	Steroid	Pharmaceutical, Veterinary Agent
Diethylhexyl phthalate	117-81-7	330.2	Y	+-	0.285 mg/L @ 24° C	-	7.6	\$26	Phthalic Acid	Pesticide Intermediate, Plasticizer

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Agonist Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Progesterone	57-83-0	314.5	Y	+-	8.8 mg/L @ 25 °C	-	3.87	\$25	Steroid	Pharmaceutical, Veterinary Agent
Tamoxifen	10540-29-1	371.5	Y	+-	practically insoluble	soluble	-	\$125	Hydrocarbon (Cyclic)	Pharmaceutical
Testosterone	58-22-0	288.4	N	+-	practically insoluble	soluble	-	\$26	Steroid	Pharmaceutical, Veterinary Agent
Bisphenol A	80-05-7	228.3	Y	+	120 mg/L @ 25 °C	-	3.32	\$12	Phenol	Chemical Intermediate, Flame Retardant, Fungicide
4-Cumylphenol	599-64-4	212.3	N	+	insoluble	-	-	\$24	Phenol	Chemical Intermediate
Daidzein	486-66-8	254.2	Y	+	practically insoluble	10 mg/ml	-	\$735	Flavonoid, Heterocyclic Compound	Natural Product
<i>p,p'</i> -DDE*	72-55-9	318.0	Y	+	0.04 mg/L 25 °C	-	6.51	\$94	Hydrocarbon (Halogenated)	Pesticide Intermediate
<i>o,p'</i> -DDT*	789-02-6	354.5	Y	+	0.085 mg/L @ 25 °C	-	6.79	\$714	Hydrocarbon (Halogenated)	Pesticide
Dicofol	115-32-2	370.489	Y	+	1.2 mg/L @ 24 °C	-	4.28	\$88	Hydrocarbon (Cyclic), Hydrocarbon (Halogenated)	Pesticide
Fenarimol	60168-88-9	331.2	Y	+	14 mg/L @ 25 °C	-	3.6	\$375	Heterocyclic Compound, Pyrimidine	Fungicide

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Agonist Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Genistein	446-72-0	270.2	Y	+	insoluble	50 mg/ml @ 25° C	2.84	\$943	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
Kaempferol	520-18-3	286.2	Y	+	slightly soluble	-	1.96	\$390	Flavonoid, Heterocyclic Compound	Natural Product
Kepone	143-50-0	490.6	Y	+	2.70 mg/L @ 25 °C	-	5.41	\$123	Hydrocarbon (Halogenated)	Pesticide
<i>p,p'</i> -Methoxychlor	72-43-5	345.7	Y	+	30.5 mg/L @ 25 °C	-	5.08	\$17	Hydrocarbon (Halogenated)	Pesticide, Veterinary Agent
Di - <i>n</i> -butyl phthalate	84-74-2	278.3	Y	+	-	-	-	\$34	Ester, Phthalic Acid	Cosmetic Ingredient, Industrial Chemical, Plasticizer
2,4,5-Trichlorophenoxyacetic acid	93-76-5	255.5	Y	+	278 mg/L	-	3.31	\$48	Carboxylic Acid	Herbicide
4-Androstenedione	63-05-8	286.4	N	-	57.8 mg/mL 25 °C	-	2.75	\$53	Steroid	Pharmaceutical
Atrazine	1912-24-9	215.7	Y	-	34.7 mg/L @ 26° C	183 g/kg @ 25° C	2.61	\$68	Heterocyclic Compound	Herbicide
Corticosterone	50-22-6	346.5	Y	-	199 mg/L @ 25° C	-	-	\$47	Steroid	Pharmaceutical

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Agonist Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Cyproterone acetate	427-51-0	416.9	Y	-	-	-	-	\$268	Steroid	Pharmaceutical
<b>Dibenzo[<i>a,h</i>]anthracene</b>	<b>53-70-3</b>	<b>278.4</b>	<b>Y</b>	<b>-</b>	<b>practically insoluble</b>	<b>-</b>	<b>6.5</b>	<b>\$352</b>	<b>Polycyclic Compound</b>	<b>Laboratory Chemical, Natural Product</b>
<b>Fluoranthene</b>	<b>206-44-0</b>	<b>202.3</b>	<b>Y</b>	<b>-</b>	<b>0.2 mg/L</b>	<b>soluble</b>	<b>5.16</b>	<b>\$21</b>	<b>Polycyclic Compound</b>	<b>Industrial Chemical, Laboratory Chemical, Pharmaceutical Intermediate</b>
Fluoxymestrone	76-43-7	336.4	Y	-	practically insoluble	-	2.38	\$131	Steroid	Pharmaceutical
Flutamide	13311-84-7	276.2	Y	-	9.45 mg/L @ 25 °C	soluble	3.35	\$22	Amide	Pharmaceutical, Veterinary Agent
Linuron	330-55-2	249.1	Y	-	75 mg/L @ 25 °C	-	3.2	\$124	Urea	Herbicide
Mifepristone (Mifeprex)	84371-65-3	429.6	Y	-	insoluble	-	-	\$262	Steroid	Pharmaceutical
<b>Phenobarbital</b>	<b>50-06-6</b>	<b>232.2</b>	<b>Y</b>	<b>-</b>	<b>1300 mg/L @ 25 °C</b>	<b>-</b>	<b>1.47</b>	<b>\$56</b>	<b>Heterocyclic Compound, Pyrimidine</b>	<b>Pharmaceutical, Veterinary Agent</b>
Procymidone	32809-16-8	284.1	Y	-	4.5 mg/L @ 25 °C	-	-	\$110	Polycyclic Compound	Fungicide

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Agonist Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Raloxifene <sup>i</sup>	82640-04-8	510.1	N	-	insoluble	28 mg/ml	-	\$235	Hydrocarbon (Cyclic)	Pharmaceutical
17b-Trenbolone	10161-33-8	270.4	Y	-	-	-	-	\$130	Steroid	Pharmaceutical
Vinclozolin	50471-44-8	286.1	Y	-	1000 mg/L @ 20 °C	-	3.10	\$41	Heterocyclic Compound	Fungicide
Actinomycin D	50-76-0	1255.4	Y	n.d. <sup>1</sup>	1 g/L at 37 °C	10 mg/ml	3.21 @ pH of 7.4	\$2,285	Heterocyclic Compound, Polycyclic Compound	Laboratory Chemical, Pharmaceutical, Veterinary Agent
Ammonium perchlorate	7790-98-9	117.5	N	n.d.	200 g/L at 25 °C	-	-	\$55	Amine, Onium Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical
4-Hydroxy androstenedione	566-48-3	302.4	Y	n.d.	-	-	-	\$107	Steroid	Pharmaceutical
Apomorphine	58-00-4	267.3	Y	n.d.	1.660 g/mL	-	2.3	\$428	Heterocyclic Compound	Pharmaceutical, Veterinary Agent
Bicalutamide	90357-06-5	430.4	Y	n.d.	5 mg/L	-	-	\$436	Amide	Pharmaceutical
2-sec-Butylphenol	89-72-5	150.2	N	n.d.	insoluble	-	3.27	\$16	Phenol	Chemical Intermediate, Pesticide Intermediate, Plasticizer

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Agonist Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Chrysin	480-40-0	254.24	Y	n.d.	84 mg/L @ 25 °C	-	3.52	\$60	Flavonoid, Heterocyclic Compound	Natural Product
<b>Clomiphene citrate</b>	<b>50-41-9</b>	<b>598.1</b>	<b>Y</b>	<b>n.d.</b>	<b>slightly soluble</b>	-	-	<b>\$45</b>	<b>Amine, Carboxylic Acid, Heterocyclic Compound</b>	<b>Pharmaceutical</b>
Cycloheximide	66-81-9	281.4	N	n.d.	0.00021 mg/L @ 25 °C	-	0.55	\$45	Heterocyclic Compound	Fungicide, Pharmaceutical, Veterinary Agent
Finasteride	98319-26-7	372.5	Y	n.d.	-	-	-	\$377	Steroid	Pharmaceutical
Haloperidol	52-86-8	375.9	Y	n.d.	1.4 mg/L @ 25 °C	soluble	4.3	\$54	Ketone	Pharmaceutical, Veterinary Agent
<b>Hydroxyflutamide</b>	<b>52806-53-8</b>	<b>292.2</b>	<b>N</b>	<b>n.d.</b>	<b>27.5 mg/L @ 25 °C</b>	-	<b>2.7</b>	<b>\$2,941</b>	<b>Amide</b>	<b>Pharmaceutical</b>
Ketoconazole	65277-42-1	531.4	Y	n.d.	0.087 mg/L @ 25 °C	-	4.35	\$380	Heterocyclic Compound	Pharmaceutical
Medroxyprogesterone acetate	71-58-9	386.5	Y	n.d.	practically insoluble	-	-	\$105	Steroid	Pharmaceutical
<b>Morin</b>	<b>480-16-0</b>	<b>302.2</b>	<b>Y</b>	<b>n.d.</b>	<b>250 mg/L @ 25 °C</b>	-	<b>1.54</b>	<b>\$14</b>	<b>Flavonoid, Heterocyclic Compound</b>	<b>Dye, Natural Product, Pharmaceutical Intermediate</b>
Nilutamide	63612-50-0	317.2	Y	n.d.	insoluble	-	-	\$45	Heterocyclic Compound, Imidazole	Pharmaceutical

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Agonist Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Norethynodrel	68-23-5	298.4	Y	n.d.	practically insoluble	-	3.51	\$78	Steroid	Pharmaceutical
Oxazepam	604-75-1	286.7	Y	n.d.	179 mg/L	-	2.24	\$463	Heterocyclic Compound	Pharmaceutical, Veterinary Agent
Ethyl paraben	120-47-8	166.2	Y	n.d.	885 mg/L @ 25 °C	-	2.47	\$8	Carboxylic Acid, Phenol	Pharmaceutical, Preservative
Phenolphthalin	81-90-3	320.3	Y	n.d.	175 mg/L @ 20 °C	-	3.95	\$26	Carboxylic Acid, Phenol	Dye, Laboratory Chemical
Pimozide	2062-78-4	461.6	Y	n.d.	insoluble	18 mg/mL	-	\$45	Heterocyclic Compound	Pharmaceutical
Propylthiouracil	51-52-5	170.2	Y	n.d.	1204 mg/L @ 25 °C	-	-	\$28	Heterocyclic Compound, Pyrimidine	Pharmaceutical, Veterinary Agent
Reserpine	50-55-5	608.7	Y	n.d.	73 mg/L @ 30 °C	-	-	\$76	Heterocyclic Compound, Indole	Pharmaceutical, Veterinary Agent
Sodium azide	26628-22-8	65.0	Y	n.d.	41 g/100 mL @ 15 °C	-	-	\$17	Azide, Salt (inorganic)	Chemical Intermediate, Fungicide, Herbicide
Spironolactone	52-01-7	416.6	Y	n.d.	22 mg/L @ 25 °C	soluble	2.78	\$38	Lactone, Steroid	Pharmaceutical
12-O-Tetradecanoylphorbol-13-acetate	16561-29-8	616.8	N	n.d.	-	soluble	-	\$11,200	Hydrocarbon (Cyclic)	Laboratory Chemical
L-Thyroxine	51-48-9	776.9	Y	n.d.	slightly soluble	-	-	\$65	Amino Acid	Pharmaceutical, Veterinary Agent



Substances listed in bolded text are included on the ICCVAM Minimum List of Substances for Validation of ER binding and TA Assays.

\**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

<sup>a</sup> CASRN = Chemical Abstracts Service Registry Number

<sup>b</sup> MW = Molecular Weight

<sup>c</sup>+++ Indicates that the substance was strongly active (EC<sub>50</sub> value was <0.001 μM); ++ indicates that the substance was moderately active (EC<sub>50</sub> value was between 0.001 and 0.1 μM); + indicates that the substance was weakly active (EC<sub>50</sub> value was >0.1 μM), or a positive response was reported without an EC<sub>50</sub> value. The EC<sub>50</sub> is the effective concentration that causes half-maximal activation of the receptor.

<sup>d</sup> Information on Solubility in Water, Solubility in DMSO, and log K<sub>ow</sub>, were obtained from the National Library of Medicine's ChemIDplus <http://chem.sis.nlm.nih.gov/chemidplus/>, and from manufacturer Materials Safety Data Sheets (MSDSs).

<sup>e</sup> 500 mg is the expected minimum amount of substance required per laboratory to conduct an endocrine disruptor (ED) validation study.

<sup>f</sup> Pricing information was obtained from vendors during October of 2005 and reflects the cost of 500 mg of substance, or the minimum amount sold.

<sup>g</sup> MeSH = Medical Subject Headings, information on chemical class criteria can be obtained at [www.nlm.nih.gov/MeSH](http://www.nlm.nih.gov/MeSH)

<sup>h</sup> A “-” in the fields for Solubility in Water, Solubility in DMSO or low K<sub>ow</sub> indicates that there is no data for this field.

<sup>i</sup> Raloxifene may act as an agonist in some *in vitro* systems.

<sup>j</sup> N indicates that this substance is not included on the EPA reference chemical list.

<sup>k</sup> Y indicates that this substance is included on the EPA reference chemical list.

<sup>l</sup> n.d. indicates that no relevant data were identified.

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## **Appendix B-3**

### **Revised ICCVAM List of Reference Substances for Validation of *In Vitro* ER TA Antagonist Assays Sorted by ER Antagonism and Substance Name**

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**Appendix B-3 Revised ICCVAM List of Reference Substances for Validation of *In Vitro* ER TA Antagonist Assays Sorted by ER TA Antagonism and Substance Name**

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Antagonism <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log Kow <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Flavone	525-82-6	222.2	N <sup>j</sup>	###	0.1 mg/L @ 25° C	- <sup>h</sup>	5.08	\$48	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
Raloxifene <sup>i</sup>	82640-04-8	510.1	N	###	insoluble	28 mg/ml	-	\$235	Hydrocarbon (Cyclic)	Pharmaceutical
Tamoxifen	10540-29-1	371.5	Y <sup>k</sup>	###	practically insoluble	soluble	-	\$125	Hydrocarbon (Cyclic)	Pharmaceutical
4-Hydroxytamoxifen	68047-06-3	387.5	N	###	practically insoluble	soluble	-	\$6,090	Hydrocarbon (Cyclic)	Pharmaceutical
Dibenzo[ <i>a,h</i> ]anthracene	53-70-3	278.4	Y	##	practically insoluble	-	6.5	\$352	Polycyclic Compound	Laboratory Chemical, Natural Product
<i>o,p'</i> -DDT*	789-02-6	354.5	Y	#	0.085 mg/L @ 25° C	-	6.79	\$714	Hydrocarbon (Halogenated)	Pesticide
Fenarimol	60168-88-9	331.2	Y	#	14 mg/L @ 25° C	-	3.6	\$375	Heterocyclic Compound, Pyrimidine	Fungicide
<i>p</i> -n -Nonylphenol	104-40-5	220.4	Y	#	-	-	5.76	\$192	Phenol	Chemical Intermediate
Resveratrol	501-36-0	228.2	N	#	-	-	3.08	\$226	Hydrocarbon (Cyclic)	Natural Product

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Antagonism <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log Kow <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Genistein	446-72-0	270.2	Y	#	insoluble	50 mg/ml @ 25° C	2.84	\$943	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
Apigenin	520-36-5	270.2	N	#-	183 mg/L @ 25° C	27 mg/ml @ 25° C	-	\$790	Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate
Atrazine	1912-24-9	215.7	Y	-	34.7 mg/L @ 26° C	183 g/kg @ 25° C	2.61	\$68	Heterocyclic Compound	Herbicide
Bisphenol A	80-05-7	228.3	Y	-	120 mg/L @ 25° C	-	3.32	\$12	Phenol	Chemical Intermediate, Flame Retardant, Fungicide
Corticosterone	50-22-6	346.5	Y	-	199 mg/L @ 25° C	-	-	\$47	Steroid	Pharmaceutical
Coumestrol	479-13-0	268.2	Y	-	practically insoluble	-	-	\$1,550	Heterocyclic Compound	Natural Product
Daidzein	486-66-8	254.2	Y	-	practically insoluble	10 mg/ml	-	\$735	Flavonoid, Heterocyclic Compound	Natural Product
<i>p,p'</i> -DDE*	72-55-9	318.0	Y	-	0.04 mg/L 25° C	-	6.51	\$94	Hydrocarbon (Halogenated)	Pesticide Intermediate
Dicofol	115-32-2	370.489	Y	-	1.2 mg/L @ 24° C	-	4.28	\$88	Hydrocarbon (Cyclic), Hydrocarbon (Halogenated)	Pesticide

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Antagonism <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log Kow <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Diethylstilbestrol	56-53-1	268.4	Y	-	12 mg/L @ 25° C	-	5.07	\$47	Hydrocarbon (Cyclic)	Pharmaceutical, Veterinary Agent
17a-Ethinyl estradiol	57-63-6	296.4	Y	-	insoluble	-	3.67	\$35	Steroid	Pharmaceutical, Veterinary Agent
Estrone	53-16-7	270.4	Y	-	0.003 g/100 mL @ 25° C	-	3.13	\$14	Steroid	Pharmaceutical, Veterinary Agent
Fluoranthene	206-44-0	202.3	Y	-	0.2 mg/L	soluble	5.16	\$21	Polycyclic Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical Intermediate
Fluoxymestrone	76-43-7	336.4	Y	-	practically insoluble	-	2.38	\$131	Steroid	Pharmaceutical
Kaempferol	520-18-3	286.2	Y	-	slightly soluble	-	1.96	\$390	Flavonoid, Heterocyclic Compound	Natural Product
<i>p,p'</i> -Methoxychlor	72-43-5	345.7	Y	-	30.5 mg/L @ 25° C	-	5.08	\$17	Hydrocarbon (Halogenated)	Pesticide, Veterinary Agent
Butylbenzyl phthalate	85-68-7	312.4	Y	-	2.69 mg/L @ 25° C	-	4.91	\$79	Carboxylic Acid, Phthalic Acid	Chemical Intermediate, Plasticizer
Di- <i>n</i> -butyl phthalate	84-74-2	278.3	Y	-	-	-	-	\$34	Ester, Phthalic Acid	Cosmetic Ingredient, Industrial Chemical, Plasticizer

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Antagonism <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log Kow <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Progesterone	57-83-0	314.5	Y	-	8.8 mg/L @ 25 °C	-	3.87	\$25	Steroid	Pharmaceutical, Veterinary Agent
Actinomycin D	50-76-0	1255.4	Y	n.d. <sup>1</sup>	1 g/L at 37 °C	10 mg/ml	3.21 @ pH of 7.4	\$2,285	Heterocyclic Compound, Polycyclic Compound	Laboratory Chemical, Pharmaceutical, Veterinary Agent
Ammonium perchlorate	7790-98-9	117.5	N	n.d.	200 g/L at 25 °C	-	-	\$55	Amine, Onium Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical
4-Androstenedione	63-05-8	286.4	N	n.d.	57.8 mg/mL 25 °C	-	2.75	\$53	Steroid	Pharmaceutical
4-Hydroxy androstenedione	566-48-3	302.4	Y	n.d.	-	-	-	\$107	Steroid	Pharmaceutical
Apomorphine	58-00-4	267.3	Y	n.d.	1.660 g/mL	-	2.3	\$428	Heterocyclic Compound	Pharmaceutical, Veterinary Agent
Bicalutamide	90357-06-5	430.4	Y	n.d.	5 mg/L	-	-	\$436	Amide	Pharmaceutical



Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Antagonism <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log Kow <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Bisphenol B	77-40-7	242.3	Y	n.d.	1 g in 50 mL	-	2.30	\$110	Phenol	Chemical Intermediate, Flame Retardant, Fungicide
2-sec-Butylphenol	89-72-5	150.2	N	n.d.	insoluble	-	3.27	\$16	Phenol	Chemical Intermediate, Pesticide Intermediate, Plasticizer
Chrysin	480-40-0	254.24	Y	n.d.	84 mg/L @ 25 °C	-	3.52	\$60	Flavonoid, Heterocyclic Compound	Natural Product
Clomiphene citrate	50-41-9	598.1	Y	n.d.	slightly soluble	-	-	\$45	Amine, Carboxylic Acid, Heterocyclic Compound	Pharmaceutical
12-O-Tetradecanoylphorbol-13-acetate	16561-29-8	616.8	N	n.d.	-	soluble	-	\$11,200	Hydrocarbon (Cyclic)	Chemical Intermediate
4-Cumylphenol	599-64-4	212.3	N	n.d.	insoluble	-	-	\$24	Phenol	Fungicide, Pharmaceutical, Veterinary Agent
Cycloheximide	66-81-9	281.4	N	n.d.	0.00021 mg/L @ 25 °C	-	0.55	\$45	Heterocyclic Compound	Pharmaceutical

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Antagonism <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log Kow <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Cyproterone acetate	427-51-0	416.9	Y	n.d.	-	-	-	\$268	Steroid	Pharmaceutical, Veterinary Agent
<b>Dexamethasone</b>	<b>50-02-2</b>	<b>392.5</b>	<b>Y</b>	<b>n.d.</b>	<b>10 mg/100mL @ 25 °C</b>	-	-	<b>\$352</b>	<b>Steroid</b>	<b>Pharmaceutical</b>
<b>5a-Dihydrotestosterone</b>	<b>521-18-6</b>	<b>290.4</b>	<b>Y</b>	<b>n.d.</b>	<b>practically insoluble</b>	-	<b>3.55</b>	<b>\$27</b>	<b>Steroid</b>	<b>Pharmaceutical, Veterinary Agent</b>
<b>17a-Estradiol</b>	<b>57-91-0</b>	<b>272.4</b>	<b>Y</b>	<b>n.d.</b>	<b>3.9 mg/L</b>	-	<b>3.94</b>	<b>\$230</b>	<b>Steroid</b>	<b>Pharmaceutical, Veterinary Agent</b>
<b>17β-Estradiol</b>	<b>50-28-2</b>	<b>272.4</b>	<b>Y</b>	<b>n.d.</b>	<b>3.60 mg/L @ 27 °C</b>	<b>soluble</b>	<b>4.01</b>	<b>\$151</b>	<b>Steroid</b>	<b>Pharmaceutical</b>
Finasteride	98319-26-7	372.5	Y	n.d.	-	-	-	\$377	Steroid	Pharmaceutical, Veterinary Agent
Flutamide	13311-84-7	276.2	Y	n.d.	9.45 mg/L @ 25 °C	soluble	3.35	\$22	Amide	Pharmaceutical, Veterinary Agent
Haloperidol	52-86-8	375.9	Y	n.d.	1.4 mg/L @ 25 °C	soluble	4.3	\$54	Ketone	Pharmaceutical, Veterinary Agent

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Antagonism <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log Kow <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
<i>meso</i> -Hexestrol	84-16-2	270.4	Y	n.d.	-	-	-	\$35	Steroid	Pharmaceutical
Hydroxyflutamide	52806-53-8	292.2	N	n.d.	27.5 mg/L @ 25 °C	-	2.7	\$2,941	Amide	Pesticide
Kepone	143-50-0	490.6	Y	n.d.	2.70 mg/L @ 25 °C	-	5.41	\$123	Hydrocarbon (Halogenated)	Pharmaceutical
Ketoconazole	65277-42-1	531.4	Y	n.d.	0.087 mg/L @ 25 °C	-	4.35	\$380	Heterocyclic Compound	Herbicide
Linuron	330-55-2	249.1	Y	n.d.	75 mg/L @ 25 °C	-	3.2	\$124	Urea	Pharmaceutical
Medroxyprogesterone acetate	71-58-9	386.5	Y	n.d.	practically insoluble	-	-	\$105	Steroid	Pharmaceutical
Mifepristone (Mifeprex)	84371-65-3	429.6	Y	n.d.	insoluble	-	-	\$262	Steroid	Dye, Natural Product, Pharmaceutical Intermediate
Morin	480-16-0	302.2	Y	n.d.	250 mg/L @ 25 °C	-	1.54	\$14	Flavonoid, Heterocyclic Compound	Pharmaceutical
Nilutamide	63612-50-0	317.2	Y	n.d.	insoluble	-	-	\$45	Heterocyclic Compound, Imidazole	Pharmaceutical

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Antagonism <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log Kow <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Norethynodrel	68-23-5	298.4	Y	n.d.	practically insoluble	-	3.51	\$78	Steroid	Pharmaceutical, Veterinary Agent
19-Nortestosterone	434-22-0	274.4	N	n.d.	-	-	-	\$90	Steroid	Chemical Intermediate, Pharmaceutical Intermediate
4- <i>tert</i> -Octylphenol	140-66-9	206.3	Y	n.d.	-	-	-	\$28	Phenol	Pharmaceutical, Veterinary Agent
Oxazepam	604-75-1	286.7	Y	n.d.	179 mg/L	-	2.24	\$463	Heterocyclic Compound	Pharmaceutical, Preservative
Ethyl paraben	120-47-8	166.2	Y	n.d.	885 mg/L @ 25 °C	-	2.47	\$8	Carboxylic Acid, Phenol	Pharmaceutical, Veterinary Agent
Phenobarbital	50-06-6	232.2	Y	n.d.	1300 mg/L @ 25 °C	-	1.47	\$56	Heterocyclic Compound, Pyrimidine	Dye, Laboratory Chemical
Phenolphthalin	81-90-3	320.3	Y	n.d.	175 mg/L @ 20 °C	-	3.95	\$26	Carboxylic Acid, Phenol	Pesticide Intermediate, Plasticizer
Diethylhexyl phthalate	117-81-7	330.2	Y	n.d.	0.285 mg/L @ 24 °C	-	7.6	\$26	Phthalic Acid	Pharmaceutical

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Antagonism <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log Kow <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Pimozide	2062-78-4	461.6	Y	n.d.	insoluble	18 mg/mL	-	\$45	Heterocyclic Compound	Fungicide
Procymidone	32809-16-8	284.1	Y	n.d.	4.5 mg/L @ 25 °C	-	-	\$110	Polycyclic Compound	Pharmaceutical, Veterinary Agent
<b>Propylthiouracil</b>	<b>51-52-5</b>	<b>170.2</b>	<b>Y</b>	<b>n.d.</b>	<b>1204 mg/L @ 25 °C</b>	-	-	<b>\$28</b>	<b>Heterocyclic Compound, Pyrimidine</b>	<b>Pharmaceutical, Veterinary Agent</b>
Reserpine	50-55-5	608.7	Y	n.d.	73 mg/L @ 30 °C	-	-	\$76	Heterocyclic Compound, Indole	Chemical Intermediate, Fungicide, Herbicide
<b>Sodium azide</b>	<b>26628-22-8</b>	<b>65.0</b>	<b>Y</b>	<b>n.d.</b>	<b>41 g/100 mL @ 15 °C</b>	-	-	<b>\$17</b>	<b>Azide, Salt (inorganic)</b>	<b>Pharmaceutical</b>
Spirolactone	52-01-7	416.6	Y	n.d.	22 mg/L @ 25 °C	soluble	2.78	\$38	Lactone, Steroid	Pharmaceutical, Veterinary Agent
<b>Testosterone</b>	<b>58-22-0</b>	<b>288.4</b>	<b>N</b>	<b>n.d.</b>	<b>practically insoluble</b>	<b>soluble</b>	<b>-</b>	<b>\$26</b>	<b>Steroid</b>	<b>Pharmaceutical, Veterinary Agent</b>
<b>Methyl testosterone</b>	<b>58-18-4</b>	<b>302.5</b>	<b>Y</b>	<b>n.d.</b>	<b>-</b>	<b>-</b>	<b>3.32</b>	<b>\$26</b>	<b>Steroid</b>	<b>Laboratory Chemical</b>

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	ER Antagonism <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
L-Thyroxine	51-48-9	776.9	Y	n.d.	slightly soluble	-	-	\$65	Amino Acid	Pharmaceutical, Veterinary Agent
17b-Trenbolone	10161-33-8	270.4	Y	n.d.	-	-	-	\$130	Steroid	Pharmaceutical
<b>2,4,5-Trichlorophenoxyacetic acid</b>	<b>93-76-5</b>	<b>255.5</b>	<b>Y</b>	<b>n.d.</b>	<b>278 mg/L</b>	<b>-</b>	<b>3.31</b>	<b>\$48</b>	<b>Carboxylic Acid</b>	<b>Herbicide</b>
<b>Vinclozolin</b>	<b>50471-44-8</b>	<b>286.1</b>	<b>Y</b>	<b>n.d.</b>	<b>1000 mg/L @ 20 °C</b>	<b>-</b>	<b>3.10</b>	<b>\$41</b>	<b>Heterocyclic Compound</b>	<b>Fungicide</b>

Substances listed in bolded text are included on the ICCVAM Minimum List of Substances for Validation of ER binding and TA Assays.

\**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

<sup>a</sup> CASRN = Chemical Abstracts Service Registry Number

<sup>b</sup> MW = Molecular Weight

<sup>c</sup> ### 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.

<sup>d</sup> Information on Solubility in Water, Solubility in DMSO, and log K<sub>ow</sub>, were obtained from the National Library of Medicine's ChemIDplus <http://chem.sis.nlm.nih.gov/chemidplus/>, and from manufacturer Materials Safety Data Sheets (MSDSs).

<sup>e</sup> 500 mg is the expected minimum amount of substance required per laboratory to conduct an endocrine disruptor (ED) validation study.

<sup>f</sup> Pricing information was obtained from vendors during October of 2005 and reflects the cost of 500 mg of substance, or the minimum amount sold.

<sup>g</sup> MeSH = Medical Subject Headings, information on chemical class criteria can be obtained at [www.nlm.nih.gov/MeSH](http://www.nlm.nih.gov/MeSH)

<sup>h</sup> A "--" in the fields for Solubility in Water, Solubility in DMSO or low K<sub>ow</sub> indicates that there is no data for this field.

<sup>i</sup> Raloxifene may act as an agonist in some *in vitro* systems.

<sup>j</sup> N indicates that this substance is not included on the EPA reference chemical list.

<sup>k</sup> Y indicates that this substance is included on the EPA reference chemical list.

<sup>l</sup> n.d. indicates that no relevant data were identified.

## **Appendix B-4**

### **Revised ICCVAM List of Reference Substances for Validation of *In Vitro* AR Binding Assays Sorted by AR Binding Activity and Substance Name**

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**Appendix B-4 Revised ICCVAM List of Reference Substances for Validation of *In Vitro* AR Binding Assays Sorted by AR Binding Activity and Substance Name**

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Binding Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
4-Androstenedione	63-05-8	286.4	N <sup>j</sup>	+++	57.8 mg/mL 25 °C	- <sup>h</sup>	2.75	\$53	Steroid	Pharmaceutical
Bicalutamide	90357-06-5	430.4	Y <sup>k</sup>	+++	5 mg/L	-	-	\$436	Amide	Pharmaceutical
Cyproterone acetate	427-51-0	416.9	Y	+++	-	-	-	\$268	Steroid	Pharmaceutical
5a-Dihydrotestosterone	521-18-6	290.4	Y	+++	practically insoluble	-	3.55	\$27	Steroid	Pharmaceutical
Medroxyprogesterone acetate	71-58-9	386.5	Y	+++	practically insoluble	-	-	\$105	Steroid	Pharmaceutical
Mifepristone (Mifeprex)	84371-65-3	429.6	Y	+++	insoluble	-	-	\$262	Steroid	Pharmaceutical
Nilutamide	63612-50-0	317.2	Y	+++	insoluble	-	-	\$45	Heterocyclic Compound, Imidazole	Pharmaceutical
19-Nortestosterone	434-22-0	274.4	N	+++	-	-	-	\$90	Steroid	Pharmaceutical, Veterinary Agent
Progesterone	57-83-0	314.5	Y	+++	8.8 mg/L @ 25 °C	-	3.87	\$25	Steroid	Pharmaceutical, Veterinary Agent
Spirolactone	52-01-7	416.6	Y	+++	22 mg/L @ 25 °C	soluble	2.78	\$38	Lactone, Steroid	Pharmaceutical

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Binding Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Testosterone	58-22-0	288.4	N	+++	practically insoluble	soluble	-	\$26	Steroid	Pharmaceutical, Veterinary Agent
Methyl testosterone	58-18-4	302.5	Y	+++	-	-	3.32	\$26	Steroid	Pharmaceutical, Veterinary Agent
17b-Trenbolone	10161-33-8	270.4	Y	+++	-	-	-	\$130	Steroid	Pharmaceutical
4-Hydroxy androstenedione	566-48-3	302.4	Y	++	-	-	-	\$107	Steroid	Pharmaceutical
<i>p,p'</i> -DDE*	72-55-9	318.0	Y	++	0.04 mg/L @ 25 °C	-	6.51	\$94	Hydrocarbon (Halogenated)	Pesticide Intermediate
Diethylstilbestrol	56-53-1	268.4	Y	++	12 mg/L @ 25° C	-	5.07	\$47	Hydrocarbon (Cyclic)	Pharmaceutical, Veterinary Agent
17a-Ethinyl estradiol	57-63-6	296.4	Y	++	insoluble	-	3.67	\$35	Steroid	Pharmaceutical, Veterinary Agent
17β-Estradiol	50-28-2	272.4	Y	++	3.60 mg/L @ 27 °C	soluble	4.01	\$151	Steroid	Pharmaceutical, Veterinary Agent
Estrone	53-16-7	270.4	Y	++	0.003 g/ 100 mL @ 25° C	-	3.13	\$14	Steroid	Pharmaceutical, Veterinary Agent
Fluoxymestrone	76-43-7	336.4	Y	++	practically insoluble	-	2.38	\$131	Steroid	Pharmaceutical

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Binding Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Flutamide	13311-84-7	276.2	Y	++	9.45 mg/L @ 25 °C	soluble	3.35	\$22	Amide	Pharmaceutical, Veterinary Agent
Hydroxyflutamide	52806-53-8	292.2	N	++	27.5 mg/L @ 25 °C	-	2.7	\$2,941	Amide	Pharmaceutical
Kepono	143-50-0	490.6	Y	++	2.70 mg/L @ 25 °C	-	5.41	\$123	Hydrocarbon (Halogenated)	Pesticide
Atrazine	1912-24-9	215.7	Y	+	34.7 mg/L @ 26° C	183 g/kg @ 25° C	2.61	\$68	Heterocyclic Compound	Herbicide
Corticosterone	50-22-6	346.5	Y	+	199 mg/L @ 25° C	-	-	\$47	Steroid	Pharmaceutical
<i>o,p'</i> -DDT*	789-02-6	354.5	Y	+	0.085 mg/L @ 25° C	-	6.79	\$714	Hydrocarbon (Halogenated)	Pesticide
Linuron	330-55-2	249.1	Y	+	75 mg/L @ 25 °C	-	3.2	\$124	Urea	Herbicide
<i>p,p'</i> -Methoxychlor	72-43-5	345.7	Y	+	30.5 mg/L @ 25 °C	-	5.08	\$17	Hydrocarbon (Halogenated)	Pesticide, Veterinary Agent
Procymidone	32809-16-8	284.1	Y	+	4.5 mg/L @ 25 °C	-	-	\$110	Polycyclic Compound	Fungicide
Dexamethasone	50-02-2	392.5	Y	-	10 mg/100mL @ 25 °C	-	-	\$352	Steroid	Pharmaceutical, Veterinary Agent

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Binding Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Actinomycin D	50-76-0	1255.4	Y	n.d. <sup>1</sup>	1 g/L at 37 °C	10 mg/ml	3.21 @ pH of 7.4	\$2,285	Heterocyclic Compound, Polycyclic Compound	Laboratory Chemical, Pharmaceutical, Veterinary Agent
Ammonium perchlorate	7790-98-9	117.5	N	n.d.	200 g/L at 25 °C	-	-	\$55	Amine, Onium Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical
Apigenin	520-36-5	270.2	N	n.d.	183 mg/L @ 25° C	27 mg/ml @ 25° C	-	\$790	Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate
Apomorphine	58-00-4	267.3	Y	n.d.	1.660 g/mL	-	2.3	\$428	Heterocyclic Compound	Pharmaceutical, Veterinary Agent
Bisphenol A	80-05-7	228.3	Y	n.d.	120 mg/L @ 25° C	-	3.32	\$12	Phenol	Chemical Intermediate, Flame Retardant, Fungicide
Bisphenol B	77-40-7	242.3	Y	n.d.	1 g in 50 mL	-	2.30	\$110	Phenol	Chemical Intermediate, Flame Retardant, Fungicide

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Binding Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
2- <i>sec</i> -Butylphenol	89-72-5	150.2	N	n.d.	insoluble	-	3.27	\$16	Phenol	Chemical Intermediate, Pesticide Intermediate, Plasticizer
Chrysin	480-40-0	254.24	Y	n.d.	84 mg/L @ 25 °C	-	3.52	\$60	Flavonoid, Heterocyclic Compound	Natural Product
Clomiphene citrate	50-41-9	598.1	Y	n.d.	slightly soluble	-	-	\$45	Amine, Carboxylic Acid, Heterocyclic Compound	Pharmaceutical
Coumestrol	479-13-0	268.2	Y	n.d.	practically insoluble	-	-	\$1,550	Heterocyclic Compound	Natural Product
4-Cumylphenol	599-64-4	212.3	N	n.d.	insoluble	-	-	\$24	Phenol	Chemical Intermediate
Cycloheximide	66-81-9	281.4	N	n.d.	0.00021 mg/L @ 25 °C	-	0.55	\$45	Heterocyclic Compound	Fungicide, Pharmaceutical, Veterinary Agent
Daidzein	486-66-8	254.2	Y	n.d.	practically insoluble	10 mg/ml	-	\$735	Flavonoid, Heterocyclic Compound	Natural Product

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Binding Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Dibenzo[ <i>a,h</i> ]anthracene	53-70-3	278.4	Y	n.d.	practically insoluble	-	6.5	\$352	Polycyclic Compound	Laboratory Chemical, Natural Product
<b>Dicofol</b>	<b>115-32-2</b>	<b>370.489</b>	<b>Y</b>	<b>n.d.</b>	<b>1.2 mg/L @ 24° C</b>	-	<b>4.28</b>	<b>\$88</b>	<b>Hydrocarbon (Cyclic), Hydrocarbon (Halogenated)</b>	<b>Pesticide</b>
17a-Estradiol	57-91-0	272.4	Y	n.d.	3.9 mg/L	-	3.94	\$230	Steroid	Pharmaceutical, Veterinary Agent
Fenarimol	60168-88-9	331.2	Y	n.d.	14 mg/L @ 25° C	-	3.6	\$375	Heterocyclic Compound, Pyrimidine	Fungicide
<b>Finasteride</b>	<b>98319-26-7</b>	<b>372.5</b>	<b>Y</b>	<b>n.d.</b>	-	-	-	<b>\$377</b>	<b>Steroid</b>	<b>Pharmaceutical</b>
Flavone	525-82-6	222.2	N	n.d.	0.1 mg/L @ 25° C	-	5.08	\$48	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
<b>Fluoranthene</b>	<b>206-44-0</b>	<b>202.3</b>	<b>Y</b>	<b>n.d.</b>	<b>0.2 mg/L</b>	<b>soluble</b>	<b>5.16</b>	<b>\$21</b>	<b>Polycyclic Compound</b>	<b>Industrial Chemical, Laboratory Chemical, Pharmaceutical Intermediate</b>

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Binding Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Genistein	446-72-0	270.2	Y	n.d.	insoluble	50 mg/ml @ 25° C	2.84	\$943	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
Haloperidol	52-86-8	375.9	Y	n.d.	1.4 mg/L @ 25° C	soluble	4.3	\$54	Ketone	Pharmaceutical, Veterinary Agent
<i>meso</i> -Hexestrol	84-16-2	270.4	Y	n.d.	-	-	-	\$35	Steroid	Pharmaceutical, Veterinary Agent
<b>4-Hydroxytamoxifen</b>	<b>68047-06-3</b>	<b>387.5</b>	<b>N</b>	<b>n.d.</b>	<b>practically insoluble</b>	<b>soluble</b>	<b>-</b>	<b>\$6,090</b>	<b>Hydrocarbon (Cyclic)</b>	<b>Pharmaceutical</b>
Kaempferol	520-18-3	286.2	Y	n.d.	slightly soluble	-	1.96	\$390	Flavonoid, Heterocyclic Compound	Natural Product
<b>Ketoconazole</b>	<b>65277-42-1</b>	<b>531.4</b>	<b>Y</b>	<b>n.d.</b>	<b>0.087 mg/L @ 25° C</b>	<b>-</b>	<b>4.35</b>	<b>\$380</b>	<b>Heterocyclic Compound</b>	<b>Pharmaceutical</b>
Morin	480-16-0	302.2	Y	n.d.	250 mg/L @ 25° C	-	1.54	\$14	Flavonoid, Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate
<b><i>p</i>-n -Nonylphenol</b>	<b>104-40-5</b>	<b>220.4</b>	<b>Y</b>	<b>n.d.</b>	<b>-</b>	<b>-</b>	<b>5.76</b>	<b>\$192</b>	<b>Phenol</b>	<b>Chemical Intermediate</b>
Norethynodrel	68-23-5	298.4	Y	n.d.	practically insoluble	-	3.51	\$78	Steroid	Pharmaceutical

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Binding Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
<b>4-tert-Octylphenol</b>	<b>140-66-9</b>	<b>206.3</b>	<b>Y</b>	<b>n.d.</b>	-	-	-	<b>\$28</b>	<b>Phenol</b>	<b>Chemical Intermediate, Pharmaceutical Intermediate</b>
Oxazepam	604-75-1	286.7	Y	n.d.	179 mg/L	-	2.24	\$463	Heterocyclic Compound	Pharmaceutical, Veterinary Agent
Ethyl paraben	120-47-8	166.2	Y	n.d.	885 mg/L @ 25 °C	-	2.47	\$8	Carboxylic Acid, Phenol	Pharmaceutical, Preservative
<b>Phenobarbital</b>	<b>50-06-6</b>	<b>232.2</b>	<b>Y</b>	<b>n.d.</b>	<b>1300 mg/L @ 25 °C</b>	-	<b>1.47</b>	<b>\$56</b>	<b>Heterocyclic Compound, Pyrimidine</b>	<b>Pharmaceutical, Veterinary Agent</b>
Phenolphthalin	81-90-3	320.3	Y	n.d.	175 mg/L @ 20 °C	-	3.95	\$26	Carboxylic Acid, Phenol	Dye, Laboratory Chemical
Butylbenzyl phthalate	85-68-7	312.4	Y	n.d.	2.69 mg/L @ 25° C	-	4.91	\$79	Carboxylic Acid, Phthalic Acid	Chemical Intermediate, Plasticizer
<b>Diethylhexyl phthalate</b>	<b>117-81-7</b>	<b>330.2</b>	<b>Y</b>	<b>n.d.</b>	<b>0.285 mg/L @ 24 °C</b>	-	<b>7.6</b>	<b>\$26</b>	<b>Phthalic Acid</b>	<b>Pesticide Intermediate, Plasticizer</b>
<b>Di - n -butyl phthalate</b>	<b>84-74-2</b>	<b>278.3</b>	<b>Y</b>	<b>n.d.</b>	-	-	-	<b>\$34</b>	<b>Ester, Phthalic Acid</b>	<b>Cosmetic Ingredient, Industrial Chemical, Plasticizer</b>



Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Binding Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Pimozide	2062-78-4	461.6	Y	n.d.	insoluble	18 mg/mL	-	\$45	Heterocyclic Compound	Pharmaceutical
Propylthiouracil	51-52-5	170.2	Y	n.d.	1204 mg/L @ 25 °C	-	-	\$28	Heterocyclic Compound, Pyrimidine	Pharmaceutical, Veterinary Agent
Raloxifene <sup>i</sup>	82640-04-8	510.1	N	n.d.	insoluble	28 mg/ml	-	\$235	Hydrocarbon (Cyclic)	Pharmaceutical
Reserpine	50-55-5	608.7	Y	n.d.	73 mg/L @ 30 °C	-	-	\$76	Heterocyclic Compound, Indole	Pharmaceutical, Veterinary Agent
Resveratrol	501-36-0	228.2	N	n.d.	-	-	3.08	\$226	Hydrocarbon (Cyclic)	Natural Product
<b>Sodium azide</b>	<b>26628-22-8</b>	<b>65.0</b>	<b>Y</b>	<b>n.d.</b>	<b>41 g/100 mL @ 15 °C</b>	-	-	<b>\$17</b>	<b>Azide, Salt (inorganic)</b>	<b>Chemical Intermediate, Fungicide, Herbicide</b>
Tamoxifen	10540-29-1	371.5	Y	n.d.	practically insoluble	soluble	-	\$125	Hydrocarbon (Cyclic)	Pharmaceutical
<b>12-O-Tetradecanoylphorbol-13-acetate</b>	<b>16561-29-8</b>	<b>616.8</b>	-	<b>n.d.</b>	-	<b>soluble</b>	-	<b>\$11,200</b>	<b>Hydrocarbon (Cyclic)</b>	<b>Laboratory Chemical</b>

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Binding Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
L-Thyroxine	51-48-9	776.9	Y	n.d.	slightly soluble	-	-	\$65	Amino Acid	Pharmaceutical, Veterinary Agent
<b>2,4,5-Trichlorophenoxyacetic acid</b>	<b>93-76-5</b>	<b>255.5</b>	<b>Y</b>	<b>n.d.</b>	<b>278 mg/L</b>	<b>-</b>	<b>3.31</b>	<b>\$48</b>	<b>Carboxylic Acid</b>	<b>Herbicide</b>
<b>Vinclozolin</b>	<b>50471-44-8</b>	<b>286.1</b>	<b>Y</b>	<b>n.d.</b>	<b>1000 mg/L @ 20 °C</b>	<b>-</b>	<b>3.10</b>	<b>\$41</b>	<b>Heterocyclic Compound</b>	<b>Fungicide</b>

Substances listed in bolded text are included on the ICCVAM Minimum List of Substances for Validation of AR binding and TA Assays.

\**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

<sup>a</sup> CASRN = Chemical Abstracts Service Registry Number

<sup>b</sup> MW = Molecular Weight

<sup>c</sup>+++ Indicates that the substance was strongly 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 IC<sub>50</sub> value 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 (IC<sub>50</sub>) is the concentration of test substances that displaces 50% of the radiolabeled reference estrogen or androgen from the receptor.

<sup>d</sup> Information on Solubility in Water, Solubility in DMSO, and log K<sub>ow</sub>, were obtained from the National Library of Medicine's ChemIDplus <http://chem.sis.nlm.nih.gov/chemidplus/>, and from manufacturer Materials Safety Data Sheets (MSDSs).

<sup>e</sup> 500 mg is the expected minimum amount of substance required per laboratory to conduct an endocrine disruptor (ED) validation study.

<sup>f</sup> Pricing information was obtained from vendors during October of 2005 and reflects the cost of 500 mg of substance, or the minimum amount sold.

<sup>g</sup> MeSH = Medical Subject Headings, information on chemical class criteria can be obtained at [www.nlm.nih.gov/MeSH](http://www.nlm.nih.gov/MeSH)

<sup>h</sup> A "--" in the fields for Solubility in Water, Solubility in DMSO or low K<sub>ow</sub> indicates that there is no data for this field.

<sup>i</sup> Raloxifene may act as an agonist in some *in vitro* systems.

<sup>j</sup> N indicates that this substance is not included on the EPA reference chemical list.

<sup>k</sup> Y indicates that this substance is included on the EPA reference chemical list.

<sup>l</sup> n.d. indicates that no relevant data were identified.

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## **Appendix B-5**

### **Revised ICCVAM List of Reference Substances for Validation of *In Vitro* AR TA Agonist Assays Sorted by AR Agonist Activity and Substance Name**

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**Appendix B-5 Revised ICCVAM List of Reference substances for Validation of *In Vitro* AR TA Agonist Assays Sorted by AR TA Agonist Activity and Substance Name**

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Agonist Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
5 $\alpha$ -Dihydrotestosterone	521-18-6	290.4	Y <sup>j</sup>	+++	practically insoluble	- <sup>h</sup>	3.55	\$27	Steroid	Pharmaceutical
19-Nortestosterone	434-22-0	274.4	N <sup>k</sup>	+++	-	-	-	\$90	Steroid	Pharmaceutical, Veterinary Agent
Testosterone	58-22-0	288.4	N	+++	practically insoluble	soluble	-	\$26	Steroid	Pharmaceutical, Veterinary Agent
<i>p,p'</i> -DDE*	72-55-9	318.0	Y	++	0.04 mg/L 25 °C	-	6.51	\$94	Hydrocarbon (Halogenated)	Pesticide Intermediate
17 $\beta$ -Estradiol	50-28-2	272.4	Y	++	3.60 mg/L @ 27 °C	soluble	4.01	\$151	Steroid	Pharmaceutical, Veterinary Agent
Estrone	53-16-7	270.4	Y	++	0.003 g/ 100 mL @ 25° C	-	3.13	\$14	Steroid	Pharmaceutical, Veterinary Agent
Mifepristone (Mifeprex)	84371-65-3	429.6	Y	++	insoluble	-	-	\$262	Steroid	Pharmaceutical
Methyl testosterone	58-18-4	302.5	Y	++	-	-	3.32	\$26	Steroid	Pharmaceutical, Veterinary Agent

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Agonist Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Ketoconazole	65277-42-1	531.4	Y	+-	0.087 mg/L @ 25 °C	-	4.35	\$380	Heterocyclic Compound	Pharmaceutical
Nilutamide	63612-50-0	317.2	Y	+-	insoluble	-	-	\$45	Heterocyclic Compound, Imidazole	Pharmaceutical
<i>p</i> -n -Nonylphenol	104-40-5	220.4	Y	+-	-	-	5.76	\$192	Phenol	Chemical Intermediate
Bicalutamide	90357-06-5	430.4	Y	+	5 mg/L	-	-	\$436	Amide	Pharmaceutical
Cyproterone acetate	427-51-0	416.9	Y	+	-	-	-	\$268	Steroid	Pharmaceutical
Dexamethasone	50-02-2	392.5	Y	+	10 mg/100mL @ 25 °C	-	-	\$352	Steroid	Pharmaceutical, Veterinary Agent
Dibenzo[ <i>a,h</i> ]anthracene	53-70-3	278.4	Y	+	practically insoluble	-	6.5	\$352	Polycyclic Compound	Laboratory Chemical, Natural Product
Fluoxymestron	76-43-7	336.4	Y	+	practically insoluble	-	2.38	\$131	Steroid	Pharmaceutical
Hydroxyflutamide	52806-53-8	292.2	N	+	27.5 mg/L @ 25 °C	-	2.7	\$2,941	Amide	Pharmaceutical
Linuron	330-55-2	249.1	Y	+	75 mg/L @ 25 °C	-	3.2	\$124	Urea	Herbicide
Medroxyprogesterone acetate	71-58-9	386.5	Y	+	practically insoluble	-	-	\$105	Steroid	Pharmaceutical

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Agonist Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Progesterone	57-83-0	314.5	Y	+	8.8 mg/L @ 25 °C	-	3.87	\$25	Steroid	Pharmaceutical, Veterinary Agent
Spirolactone	52-01-7	416.6	Y	+	22 mg/L @ 25 °C	soluble	2.78	\$38	Lactone, Steroid	Pharmaceutical
Apigenin	520-36-5	270.2	N	-	183 mg/L @ 25 °C	27 mg/ml @ 25 °C	-	\$790	Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate
Atrazine	1912-24-9	215.7	Y	-	34.7 mg/L @ 26 °C	183 g/kg @ 25 °C	2.61	\$68	Heterocyclic Compound	Herbicide
Bisphenol A	80-05-7	228.3	Y	-	120 mg/L @ 25 °C	-	3.32	\$12	Phenol	Chemical Intermediate, Flame Retardant, Fungicide
Bisphenol B	77-40-7	242.3	Y	-	1 g in 50 mL	-	2.30	\$110	Phenol	Chemical Intermediate, Flame Retardant, Fungicide
Corticosterone	50-22-6	346.5	Y	-	199 mg/L @ 25 °C	-	-	\$47	Steroid	Pharmaceutical
Coumestrol	479-13-0	268.2	Y	-	practically insoluble	-	-	\$1,550	Heterocyclic Compound	Natural Product
4-Cumylphenol	599-64-4	212.3	N	-	insoluble	-	-	\$24	Phenol	Chemical Intermediate



Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Agonist Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
<i>o,p'</i> -DDT*	789-02-6	354.5	Y	-	0.085 mg/L @ 25° C	-	6.79	\$714	Hydrocarbon (Halogenated)	Pesticide
Diethylstilbestrol	56-53-1	268.4	Y	-	12 mg/L @ 25° C	-	5.07	\$47	Hydrocarbon (Cyclic)	Pharmaceutical, Veterinary Agent
17 $\alpha$ -Estradiol	57-91-0	272.4	Y	-	3.9 mg/L	-	3.94	\$230	Steroid	Pharmaceutical, Veterinary Agent
17 $\alpha$ -Ethinyl estradiol	57-63-6	296.4	Y	-	insoluble	-	3.67	\$35	Steroid	Pharmaceutical, Veterinary Agent
Finasteride	98319-26-7	372.5	Y	-	-	-	-	\$377	Steroid	Pharmaceutical
Flutamide	13311-84-7	276.2	Y	-	9.45 mg/L @ 25 °C	soluble	3.35	\$22	Amide	Pharmaceutical, Veterinary Agent
4-Hydroxytamoxifen	68047-06-3	387.5	N	-	practically insoluble	soluble	-	\$6,090	Hydrocarbon (Cyclic)	Pharmaceutical
Kepone	143-50-0	490.6	Y	-	2.70 mg/L @ 25 °C	-	5.41	\$123	Hydrocarbon (Halogenated)	Pesticide
<i>p,p'</i> -Methoxychlor	72-43-5	345.7	Y	-	30.5 mg/L @ 25 °C	-	5.08	\$17	Hydrocarbon (Halogenated)	Pesticide, Veterinary Agent

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Agonist Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
4- <i>tert</i> -Octylphenol	140-66-9	206.3	Y	-	-	-	-	\$28	Phenol	Chemical Intermediate, Pharmaceutical Intermediate
Phenobarbital	50-06-6	232.2	Y	-	1300 mg/L @ 25 °C	-	1.47	\$56	Heterocyclic Compound, Pyrimidine	Pharmaceutical, Veterinary Agent
Butylbenzyl phthalate	85-68-7	312.4	Y	-	2.69 mg/L @ 25° C	-	4.91	\$79	Carboxylic Acid, Phthalic Acid	Chemical Intermediate, Plasticizer
Diethylhexyl phthalate	117-81-7	330.2	Y	-	0.285 mg/L @ 24 °C	-	7.6	\$26	Phthalic Acid	Pesticide Intermediate, Plasticizer
Di - <i>n</i> -butyl phthalate	84-74-2	278.3	Y	-	-	-	-	\$34	Ester, Phthalic Acid	Cosmetic Ingredient, Industrial Chemical, Plasticizer
Procymidone	32809-16-8	284.1	Y	-	4.5 mg/L @ 25 °C	-	-	\$110	Polycyclic Compound	Fungicide
Tamoxifen	10540-29-1	371.5	Y	-	practically insoluble	soluble	-	\$125	Hydrocarbon (Cyclic)	Pharmaceutical

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Agonist Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Vinclozolin	50471-44-8	286.1	Y	-	1000 mg/L @ 20 °C	-	3.10	\$41	Heterocyclic Compound	Fungicide
Actinomycin D	50-76-0	1255.4	Y	n.d. <sup>i</sup>	1 g/L at 37 °C	10 mg/ml	3.21 @ pH of 7.4	\$2,285	Heterocyclic Compound, Polycyclic Compound	Laboratory Chemical, Pharmaceutical, Veterinary Agent
Ammonium perchlorate	7790-98-9	117.5	N	n.d.	200 g/L at 25 °C	-	-	\$55	Amine, Onium Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical
4-Androstenedione	63-05-8	286.4	N	n.d.	57.8 mg/mL 25 °C	-	2.75	\$53	Steroid	Pharmaceutical
4-Hydroxy androstenedione	566-48-3	302.4	Y	n.d.	-	-	-	\$107	Steroid	Pharmaceutical
Apomorphine	58-00-4	267.3	Y	n.d.	1.660 g/mL	-	2.3	\$428	Heterocyclic Compound	Pharmaceutical, Veterinary Agent
2-sec-Butylphenol	89-72-5	150.2	N	n.d.	insoluble	-	3.27	\$16	Phenol	Chemical Intermediate, Pesticide Intermediate, Plasticizer

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Agonist Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Chrysin	480-40-0	254.24	Y	n.d.	84 mg/L @ 25 °C	-	3.52	\$60	Flavonoid, Heterocyclic Compound	Natural Product
Clomiphene citrate	50-41-9	598.1	Y	n.d.	slightly soluble	-	-	\$45	Amine, Carboxylic Acid, Heterocyclic Compound	Pharmaceutical
Cycloheximide	66-81-9	281.4	N	n.d.	0.00021 mg/L @ 25 °C	-	0.55	\$45	Heterocyclic Compound	Fungicide, Pharmaceutical, Veterinary Agent
Daidzein	486-66-8	254.2	Y	n.d.	practically insoluble	10 mg/ml	-	\$735	Flavonoid, Heterocyclic Compound	Natural Product
<b>Dicofol</b>	<b>115-32-2</b>	<b>370.489</b>	<b>Y</b>	<b>n.d.</b>	<b>1.2 mg/L @ 24°C</b>	<b>-</b>	<b>4.28</b>	<b>\$88</b>	<b>Hydrocarbon (Cyclic), Hydrocarbon (Halogenated)</b>	<b>Pesticide</b>
Fenarimol	60168-88-9	331.2	Y	n.d.	14 mg/L @ 25° C	-	3.6	\$375	Heterocyclic Compound, Pyrimidine	Fungicide
Flavone	525-82-6	222.2	N	n.d.	0.1 mg/L @ 25° C	-	5.08	\$48	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
<b>Fluoranthene</b>	<b>206-44-0</b>	<b>202.3</b>	<b>Y</b>	<b>n.d.</b>	<b>0.2 mg/L</b>	<b>soluble</b>	<b>5.16</b>	<b>\$21</b>	<b>Polycyclic Compound</b>	<b>Industrial Chemical, Laboratory Chemical, Pharmaceutical Intermediate</b>

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Agonist Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Genistein	446-72-0	270.2	Y	n.d.	insoluble	50 mg/ml @ 25° C	2.84	\$943	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
Haloperidol	52-86-8	375.9	Y	n.d.	1.4 mg/L @ 25 °C	soluble	4.3	\$54	Ketone	Pharmaceutical, Veterinary Agent
<i>meso</i> -Hexestrol	84-16-2	270.4	Y	n.d.	-	-	-	\$35	Steroid	Pharmaceutical, Veterinary Agent
Kaempferol	520-18-3	286.2	Y	n.d.	slightly soluble	-	1.96	\$390	Flavonoid, Heterocyclic Compound	Natural Product
Morin	480-16-0	302.2	Y	n.d.	250 mg/L @ 25 °C	-	1.54	\$14	Flavonoid, Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate
Norethynodrel	68-23-5	298.4	Y	n.d.	practically insoluble	-	3.51	\$78	Steroid	Pharmaceutical
Oxazepam	604-75-1	286.7	Y	n.d.	179 mg/L	-	2.24	\$463	Heterocyclic Compound	Pharmaceutical, Veterinary Agent
Ethyl paraben	120-47-8	166.2	Y	n.d.	885 mg/L @ 25 °C	-	2.47	\$8	Carboxylic Acid, Phenol	Pharmaceutical, Preservative
Phenolphthalin	81-90-3	320.3	Y	n.d.	175 mg/L @ 20 °C	-	3.95	\$26	Carboxylic Acid, Phenol	Dye, Laboratory Chemical
Pimozide	2062-78-4	461.6	Y	n.d.	insoluble	18 mg/mL	-	\$45	Heterocyclic Compound	Pharmaceutical

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Agonist Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Propylthiouracil	51-52-5	170.2	Y	n.d.	1204 mg/L @ 25 °C	-	-	\$28	Heterocyclic Compound, Pyrimidine	Pharmaceutical, Veterinary Agent
Raloxifene <sup>1</sup>	82640-04-8	510.1	N	n.d.	insoluble	28 mg/ml	-	\$235	Hydrocarbon (Cyclic)	Pharmaceutical
Reserpine	50-55-5	608.7	Y	n.d.	73 mg/L @ 30 °C	-	-	\$76	Heterocyclic Compound, Indole	Pharmaceutical, Veterinary Agent
Resveratrol	501-36-0	228.2	N	n.d.	-	-	3.08	\$226	Hydrocarbon (Cyclic)	Natural Product
<b>Sodium azide</b>	<b>26628-22-8</b>	<b>65.0</b>	<b>Y</b>	<b>n.d.</b>	<b>41 g/100 mL @ 15 °C</b>	-	-	<b>\$17</b>	<b>Azide, Salt (inorganic)</b>	<b>Chemical Intermediate, Fungicide, Herbicide</b>
<b>12-O-Tetradecanoylphorbol-13-acetate</b>	<b>16561-29-8</b>	<b>616.8</b>	-	<b>n.d.</b>	-	<b>soluble</b>	-	<b>\$11,200</b>	<b>Hydrocarbon (Cyclic)</b>	<b>Laboratory Chemical</b>
L-Thyroxine	51-48-9	776.9	Y	n.d.	slightly soluble	-	-	\$65	Amino Acid	Pharmaceutical, Veterinary Agent
<b>17b-Trenbolone</b>	<b>10161-33-8</b>	<b>270.4</b>	<b>Y</b>	<b>n.d.</b>	-	-	-	<b>\$130</b>	<b>Steroid</b>	<b>Pharmaceutical</b>

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Agonist Activity <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO <sup>d</sup>	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
<b>2,4,5-Trichlorophenoxyacetic acid</b>	<b>93-76-5</b>	<b>255.5</b>	<b>Y</b>	<b>n.d.</b>	<b>278 mg/L</b>	<b>-</b>	<b>3.31</b>	<b>\$48</b>	<b>Carboxylic Acid</b>	<b>Herbicide</b>

Substances listed in bolded text are included on the ICCVAM Minimum List of Substances for Validation of AR binding and TA Assays.

\**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

<sup>a</sup> CASRN = Chemical Abstracts Service Registry Number

<sup>b</sup> MW = Molecular Weight

<sup>c</sup>+++ Indicates that the substance was strongly active (EC<sub>50</sub> value was <0.001 μM); ++ indicates that the substance was moderately active (EC<sub>50</sub> value was between 0.001 and 0.1 μM); + indicates that the substance was weakly active (EC<sub>50</sub> value was >0.1 μM), or a positive response was reported without an EC<sub>50</sub> value. The EC<sub>50</sub> is the effective concentration that causes half-maximal activation of the receptor.

<sup>d</sup> Information on Solubility in Water, Solubility in DMSO, and log K<sub>ow</sub>, were obtained from the National Library of Medicine's ChemIDplus <http://chem.sis.nlm.nih.gov/chemidplus/>, and from manufacturer Materials Safety Data Sheets (MSDSs).

<sup>e</sup> 500 mg is the expected minimum amount of substance required per laboratory to conduct an endocrine disruptor (ED) validation study.

<sup>f</sup> Pricing information was obtained from vendors during October of 2005 and reflects the cost of 500 mg of substance, or the minimum amount sold.

<sup>g</sup> MeSH = Medical Subject Headings, information on chemical class criteria can be obtained at [www.nlm.nih.gov/MeSH](http://www.nlm.nih.gov/MeSH)

<sup>h</sup> A “-“ in the fields for Solubility in Water, Solubility in DMSO or low K<sub>ow</sub> indicates that there is no data for this field.

<sup>i</sup> Raloxifene may act as an agonist in some *in vitro* systems.

<sup>j</sup> Y indicates that this substance is included on the EPA reference chemical list.

<sup>k</sup> N indicates that this substance is not included on the EPA reference chemical list.

<sup>l</sup> n.d. indicates that no relevant data were identified.

## **Appendix B-6**

### **Revised ICCVAM List of Reference Substances for Validation of *In Vitro* AR TA Antagonist Assays Sorted by AR Antagonism and Substance Name**



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**Appendix B-6 Revised ICCVAM List of Reference Substances for Validation of *In Vitro* AR TA Antagonist Assays Sorted by AR TA Antagonism and Substance Name**

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Antagonism <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
<i>p</i> -n -Nonylphenol	104-40-5	220.4	Y <sup>j</sup>	###	-	- <sup>h</sup>	5.76	\$192	Phenol	Chemical Intermediate
Vinclozolin	50471-44-8	286.1	Y	###	1000 mg/L @ 20 °C	-	3.10	\$41	Heterocyclic Compound	Fungicide
Bicalutamide	90357-06-5	430.4	Y	##	5 mg/L	-	-	\$436	Amide	Pharmaceutical
Cyproterone acetate	427-51-0	416.9	Y	##	-	-	-	\$268	Steroid	Pharmaceutical
Hydroxyflutamide	52806-53-8	292.2	N <sup>k</sup>	##	27.5 mg/L @ 25 °C	-	2.7	\$2,941	Amide	Pharmaceutical
Nilutamide	63612-50-0	317.2	Y	##	insoluble	-	-	\$45	Heterocyclic Compound, Imidazole	Pharmaceutical
Spirolactone	52-01-7	416.6	Y	##	22 mg/L @ 25 °C	soluble	2.78	\$38	Lactone, Steroid	Pharmaceutical

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Antagonism <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO	log <sub>K<sub>ow</sub></sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Bisphenol A	80-05-7	228.3	Y	#-	120 mg/L @ 25° C	-	3.32	\$12	Phenol	Chemical Intermediate, Flame Retardant, Fungicide
<i>p,p'</i> -DDE*	72-55-9	318.0	Y	#-	0.04 mg/L 25° C	-	6.51	\$94	Hydrocarbon (Halogenated)	Pesticide Intermediate
Kepono	143-50-0	490.6	Y	#-	2.70 mg/L @ 25° C	-	5.41	\$123	Hydrocarbon (Halogenated)	Pesticide
Progesterone	57-83-0	314.5	Y	#-	8.8 mg/L @ 25° C	-	3.87	\$25	Steroid	Pharmaceutical, Veterinary Agent
Bisphenol B	77-40-7	242.3	Y	#	1 g in 50 mL	-	2.30	\$110	Phenol	Chemical Intermediate, Flame Retardant, Fungicide
<i>o,p'</i> -DDT*	789-02-6	354.5	Y	#	0.085 mg/L @ 25° C	-	6.79	\$714	Hydrocarbon (Halogenated)	Pesticide
Diethylstilbestrol	56-53-1	268.4	Y	#	12 mg/L @ 25° C	-	5.07	\$47	Hydrocarbon (Cyclic)	Pharmaceutical, Veterinary Agent
Fluoranthene	206-44-0	202.3	Y	#	0.2 mg/L	soluble	5.16	\$21	Polycyclic Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical Intermediate

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Antagonism <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO	log <sub>K<sub>ow</sub></sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Flutamide	13311-84-7	276.2	Y	#	9.45 mg/L @ 25 °C	soluble	3.35	\$22	Amide	Pharmaceutical, Veterinary Agent
Genistein	446-72-0	270.2	Y	#	insoluble	50 mg/ml @ 25° C	2.84	\$943	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
<b>Linuron</b>	<b>330-55-2</b>	<b>249.1</b>	<b>Y</b>	<b>#</b>	<b>75 mg/L @ 25 °C</b>	-	<b>3.2</b>	<b>\$124</b>	<b>Urea</b>	<b>Herbicide</b>
<i>p,p'</i> -Methoxychlor	72-43-5	345.7	Y	#	30.5 mg/L @ 25 °C	-	5.08	\$17	Hydrocarbon (Halogenated)	Pesticide, Veterinary Agent
Procymidone	32809-16-8	284.1	Y	#	4.5 mg/L @ 25 °C	-	-	\$110	Polycyclic Compound	Fungicide
Resveratrol	501-36-0	228.2	N	#	-	-	3.08	\$226	Hydrocarbon (Cyclic)	Natural Product
Atrazine	1912-24-9	215.7	Y	-	34.7 mg/L @ 26° C	183 g/kg @ 25° C	2.61	\$68	Heterocyclic Compound	Herbicide
Finasteride	98319-26-7	372.5	Y	-	-	-	-	\$377	Steroid	Pharmaceutical
Fluoxymestrone	76-43-7	336.4	Y	-	practically insoluble	-	2.38	\$131	Steroid	Pharmaceutical

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Antagonism <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO	log <sub>K<sub>ow</sub></sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Ketoconazole	65277-42-1	531.4	Y	-	0.087 mg/L @ 25 °C	-	4.35	\$380	Heterocyclic Compound	Pharmaceutical
Butylbenzyl phthalate	85-68-7	312.4	Y	-	2.69 mg/L @ 25 °C	-	4.91	\$79	Carboxylic Acid, Phthalic Acid	Chemical Intermediate, Plasticizer
Testosterone	58-22-0	288.4	N	-	practically insoluble	soluble	-	\$26	Steroid	Pharmaceutical, Veterinary Agent
Actinomycin D	50-76-0	1255.4	Y	n.d. <sup>1</sup>	1 g/L at 37 °C	10 mg/ml	3.21 @ pH of 7.4	\$2,285	Heterocyclic Compound, Polycyclic Compound	Laboratory Chemical, Pharmaceutical, Veterinary Agent
Ammonium perchlorate	7790-98-9	117.5	N	n.d.	200 g/L at 25 °C	-	-	\$55	Amine, Onium Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical
4-Androstenedione	63-05-8	286.4	N	n.d.	57.8 mg/mL 25 °C	-	2.75	\$53	Steroid	Pharmaceutical
4-Hydroxy androstenedione	566-48-3	302.4	Y	n.d.	-	-	-	\$107	Steroid	Pharmaceutical

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Antagonism <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO	log <sub>K<sub>ow</sub></sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Apigenin	520-36-5	270.2	N	n.d.	183 mg/L @ 25° C	27 mg/ml @ 25° C	-	\$790	Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate
Apomorphine	58-00-4	267.3	Y	n.d.	1.660 g/mL	-	2.3	\$428	Heterocyclic Compound	Pharmaceutical, Veterinary Agent
2-sec-Butylphenol	89-72-5	150.2	N	n.d.	insoluble	-	3.27	\$16	Phenol	Chemical Intermediate, Pesticide Intermediate, Plasticizer
Chrysin	480-40-0	254.24	Y	n.d.	84 mg/L @ 25° C	-	3.52	\$60	Flavonoid, Heterocyclic Compound	Natural Product
Clomiphene citrate	50-41-9	598.1	Y	n.d.	slightly soluble	-	-	\$45	Amine, Carboxylic Acid, Heterocyclic Compound	Pharmaceutical
<b>Corticosterone</b>	<b>50-22-6</b>	<b>346.5</b>	<b>Y</b>	<b>n.d.</b>	<b>199 mg/L @ 25° C</b>	<b>-</b>	<b>-</b>	<b>\$47</b>	<b>Steroid</b>	<b>Pharmaceutical</b>
Coumestrol	479-13-0	268.2	Y	n.d.	practically insoluble	-	-	\$1,550	Heterocyclic Compound	Natural Product
4-Cumylphenol	599-64-4	212.3	N	n.d.	insoluble	-	-	\$24	Phenol	Chemical Intermediate
Cycloheximide	66-81-9	281.4	N	n.d.	0.00021 mg/L @ 25° C	-	0.55	\$45	Heterocyclic Compound	Fungicide, Pharmaceutical, Veterinary Agent

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Antagonism <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO	log <sub>K<sub>ow</sub></sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Daidzein	486-66-8	254.2	Y	n.d.	practically insoluble	10 mg/ml	-	\$735	Flavonoid, Heterocyclic Compound	Natural Product
<b>Dexamethasone</b>	<b>50-02-2</b>	<b>392.5</b>	<b>Y</b>	<b>n.d.</b>	<b>10 mg/100mL @ 25 °C</b>	-	-	<b>\$352</b>	<b>Steroid</b>	<b>Pharmaceutical, Veterinary Agent</b>
Dibenzo[ <i>a,h</i> ]anthracene	53-70-3	278.4	Y	n.d.	practically insoluble	-	6.5	\$352	Polycyclic Compound	Laboratory Chemical, Natural Product
<b>Dicofol</b>	<b>115-32-2</b>	<b>370.489</b>	<b>Y</b>	<b>n.d.</b>	<b>1.2 mg/L @ 24°C</b>	-	<b>4.28</b>	<b>\$88</b>	<b>Hydrocarbon (Cyclic), Hydrocarbon (Halogenated)</b>	<b>Pesticide</b>
<b>5<math>\alpha</math>-Dihydrotestosterone</b>	<b>521-18-6</b>	<b>290.4</b>	<b>Y</b>	<b>n.d.</b>	<b>practically insoluble</b>	-	<b>3.55</b>	<b>\$27</b>	<b>Steroid</b>	<b>Pharmaceutical</b>
17 $\alpha$ -Estradiol	57-91-0	272.4	Y	n.d.	3.9 mg/L	-	3.94	\$230	Steroid	Pharmaceutical, Veterinary Agent
<b>17<math>\alpha</math>-Ethinyl estradiol</b>	<b>57-63-6</b>	<b>296.4</b>	<b>Y</b>	<b>n.d.</b>	<b>insoluble</b>	-	<b>3.67</b>	<b>\$35</b>	<b>Steroid</b>	<b>Pharmaceutical, Veterinary Agent</b>
<b>17<math>\beta</math>-Estradiol</b>	<b>50-28-2</b>	<b>272.4</b>	<b>Y</b>	<b>n.d.</b>	<b>3.60 mg/L @ 27 °C</b>	<b>soluble</b>	<b>4.01</b>	<b>\$151</b>	<b>Steroid</b>	<b>Pharmaceutical, Veterinary Agent</b>
<b>Estrone</b>	<b>53-16-7</b>	<b>270.4</b>	<b>Y</b>	<b>n.d.</b>	<b>0.003 g/100 mL @ 25° C</b>	-	<b>3.13</b>	<b>\$14</b>	<b>Steroid</b>	<b>Pharmaceutical, Veterinary Agent</b>
Fenarimol	60168-88-9	331.2	Y	n.d.	14 mg/L @ 25° C	-	3.6	\$375	Heterocyclic Compound, Pyrimidine	Fungicide

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Antagonism <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Flavone	525-82-6	222.2	N	n.d.	0.1 mg/L @ 25 °C	-	5.08	\$48	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
Haloperidol	52-86-8	375.9	Y	n.d.	1.4 mg/L @ 25 °C	soluble	4.3	\$54	Ketone	Pharmaceutical, Veterinary Agent
<i>meso</i> -Hexestrol	84-16-2	270.4	Y	n.d.	-	-	-	\$35	Steroid	Pharmaceutical, Veterinary Agent
<b>4-Hydroxytamoxifen</b>	<b>68047-06-3</b>	<b>387.5</b>	<b>N</b>	<b>n.d.</b>	<b>practically insoluble</b>	<b>soluble</b>	<b>-</b>	<b>\$6,090</b>	<b>Hydrocarbon (Cyclic)</b>	<b>Pharmaceutical</b>
Kaempferol	520-18-3	286.2	Y	n.d.	slightly soluble	-	1.96	\$390	Flavonoid, Heterocyclic Compound	Natural Product
<b>Medroxyprogesterone acetate</b>	<b>71-58-9</b>	<b>386.5</b>	<b>Y</b>	<b>n.d.</b>	<b>practically insoluble</b>	<b>-</b>	<b>-</b>	<b>\$105</b>	<b>Steroid</b>	<b>Pharmaceutical</b>
Morin	480-16-0	302.2	Y	n.d.	250 mg/L @ 25 °C	-	1.54	\$14	Flavonoid, Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate
Norethynodrel	68-23-5	298.4	Y	n.d.	practically insoluble	-	3.51	\$78	Steroid	Pharmaceutical
<b>19-Nortestosterone</b>	<b>434-22-0</b>	<b>274.4</b>	<b>N</b>	<b>n.d.</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>\$90</b>	<b>Steroid</b>	<b>Pharmaceutical, Veterinary Agent</b>
<b>4-tert-Octylphenol</b>	<b>140-66-9</b>	<b>206.3</b>	<b>Y</b>	<b>n.d.</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>\$28</b>	<b>Phenol</b>	<b>Chemical Intermediate, Pharmaceutical Intermediate</b>
Oxazepam	604-75-1	286.7	Y	n.d.	179 mg/L	-	2.24	\$463	Heterocyclic Compound	Pharmaceutical, Veterinary Agent



Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Antagonism <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Ethyl paraben	120-47-8	166.2	Y	n.d.	885 mg/L @ 25 °C	-	2.47	\$8	Carboxylic Acid, Phenol	Pharmaceutical, Preservative
<b>Phenobarbital</b>	<b>50-06-6</b>	<b>232.2</b>	<b>Y</b>	<b>n.d.</b>	<b>1300 mg/L @ 25 °C</b>	-	<b>1.47</b>	<b>\$56</b>	<b>Heterocyclic Compound, Pyrimidine</b>	<b>Pharmaceutical, Veterinary Agent</b>
Phenolphthalin	81-90-3	320.3	Y	n.d.	175 mg/L @ 20 °C	-	3.95	\$26	Carboxylic Acid, Phenol	Dye, Laboratory Chemical
<b>Diethylhexyl phthalate</b>	<b>117-81-7</b>	<b>330.2</b>	<b>Y</b>	<b>n.d.</b>	<b>0.285 mg/L @ 24 °C</b>	-	<b>7.6</b>	<b>\$26</b>	<b>Phthalic Acid</b>	<b>Pesticide Intermediate, Plasticizer</b>
<b>Di - n -butyl phthalate</b>	<b>84-74-2</b>	<b>278.3</b>	<b>Y</b>	<b>n.d.</b>	-	-	-	<b>\$34</b>	<b>Ester, Phthalic Acid</b>	<b>Cosmetic Ingredient, Industrial Chemical, Plasticizer</b>
Pimozide	2062-78-4	461.6	Y	n.d.	insoluble	18 mg/mL	-	\$45	Heterocyclic Compound	Pharmaceutical
Propylthiouracil	51-52-5	170.2	Y	n.d.	1204 mg/L @ 25 °C	-	-	\$28	Heterocyclic Compound, Pyrimidine	Pharmaceutical, Veterinary Agent
Raloxifene <sup>i</sup>	82640-04-8	510.1	N	n.d.	insoluble	28 mg/ml	-	\$235	Hydrocarbon (Cyclic)	Pharmaceutical
Reserpine	50-55-5	608.7	Y	n.d.	73 mg/L @ 30 °C	-	-	\$76	Heterocyclic Compound, Indole	Pharmaceutical, Veterinary Agent
<b>Sodium azide</b>	<b>26628-22-8</b>	<b>65.0</b>	<b>Y</b>	<b>n.d.</b>	<b>41 g/100 mL @ 15 °C</b>	-	-	<b>\$17</b>	<b>Azide, Salt (inorganic)</b>	<b>Chemical Intermediate, Fungicide, Herbicide</b>

Substance	CASRN <sup>a</sup>	MW <sup>b</sup>	EPA Reference Chemicals	AR Antagonism <sup>c</sup>	Solubility in Water <sup>d</sup>	Solubility in DMSO	log K <sub>ow</sub> <sup>d</sup>	Total Cost Per 500mg <sup>e,f</sup>	MESH Chemical Class <sup>g</sup>	Product Class
Tamoxifen	10540-29-1	371.5	Y	n.d.	practically insoluble	soluble	-	\$125	Hydrocarbon (Cyclic)	Pharmaceutical
<b>Methyl testosterone</b>	<b>58-18-4</b>	<b>302.5</b>	<b>Y</b>	<b>n.d.</b>	-	-	<b>3.32</b>	<b>\$26</b>	<b>Steroid</b>	<b>Pharmaceutical, Veterinary Agent</b>
<b>12-O-Tetradecanoylphorbol-13-acetate</b>	<b>16561-29-8</b>	<b>616.8</b>	-	<b>n.d.</b>	-	<b>soluble</b>	-	<b>\$11,200</b>	<b>Hydrocarbon (Cyclic)</b>	<b>Laboratory Chemical</b>
L-Thyroxine	51-48-9	776.9	Y	n.d.	slightly soluble	-	-	\$65	Amino Acid	Pharmaceutical, Veterinary Agent
<b>17b-Trenbolone</b>	<b>10161-33-8</b>	<b>270.4</b>	<b>Y</b>	<b>n.d.</b>	-	-	-	<b>\$130</b>	<b>Steroid</b>	<b>Pharmaceutical</b>
<b>2,4,5-Trichlorophenoxyacetic acid</b>	<b>93-76-5</b>	<b>255.5</b>	<b>Y</b>	<b>n.d.</b>	<b>278 mg/L</b>	-	<b>3.31</b>	<b>\$48</b>	<b>Carboxylic Acid</b>	<b>Herbicide</b>

Substances listed in bolded text are included on the ICCVAM Minimum List of Substances for Validation of AR binding and TA Assays.

\**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

<sup>a</sup> CASRN = Chemical Abstracts Service Registry Number

<sup>b</sup> MW = Molecular Weight

<sup>c</sup> ### 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.

<sup>d</sup> Information on Solubility in Water, Solubility in DMSO, and log K<sub>ow</sub>, were obtained from the National Library of Medicine's ChemIDplus <http://chem.sis.nlm.nih.gov/chemidplus/>, and from manufacturer Materials Safety Data Sheets (MSDSs).

<sup>e</sup> 500 mg is the expected minimum amount of substance required per laboratory to conduct an endocrine disruptor (ED) validation study.

<sup>f</sup> Pricing information was obtained from vendors during October of 2005 and reflects the cost of 500 mg of substance, or the minimum amount sold.

<sup>g</sup> MeSH = Medical Subject Headings, information on chemical class criteria can be obtained at [www.nlm.nih.gov/MeSH](http://www.nlm.nih.gov/MeSH)

<sup>h</sup> A "--" in the fields for Solubility in Water, Solubility in DMSO or low K<sub>ow</sub> indicates that there is no data for this field.

<sup>i</sup> Raloxifene may act as an agonist in some *in vitro* systems.

<sup>j</sup> Y indicates that this substance is included on the EPA reference chemical list.

<sup>k</sup> N indicates that this substance is not included on the EPA reference chemical list.

<sup>l</sup> n.d. indicates that no relevant data were identified.

## Appendix C

### ***Federal Register* Notices Regarding the Revised ICCVAM List of Reference Substances for Validation of *In Vitro* Binding and TA Assays**

- C-1 **71FR13597** Notice of Availability of a Revised List of Recommended Reference Substances for Validation of In Vitro Estrogen and Androgen Receptor Binding and Transcriptional Activation Assays: Request for Comments and Submission of In Vivo and In Vitro Data ..... C-3
- C-2 **71FR56997** Notice of Availability of a Revised List of Recommended Reference Substances for Validation of In Vitro Estrogen and Androgen Receptor Binding and Transcriptional Activation Assays ..... C-9

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## **Appendix C-1**

**71FR13597 Notice of Availability of a Revised List of Recommended Reference Substances for Validation of In Vitro Estrogen and Androgen Receptor Binding and Transcriptional Activation Assays:  
Request for Comments and Submission of In Vivo and In Vitro Data**

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hearing together with a statement setting forth in detail the facts to be proved, the relevance of those facts to the issues in this proceeding, a description of the evidence which would be adduced, and why such evidence cannot be submitted by affidavit;

(b) Should any party believe that an oral argument is required, that party must submit a request specifying the reasons therefore and why argument by memorandum is inadequate to present the party's case; and

(c) Any request for evidentiary hearing or oral argument shall be filed no later than May 17, 2006;

It is further ordered that notice of this Order to Show Cause be published in the **Federal Register**, and that a copy thereof be served upon each respondent at its last known address;

It is further ordered that all documents submitted by any party of record in this proceeding shall be filed in accordance with Rule 118 of the Commission's Rules of Practice and Procedure, 46 CFR 502.118, and be mailed directly to all parties of record;

Finally, it is ordered that pursuant to the terms of Rule 61 of the Commission's Rules of Practice and Procedure, 46 CFR 502.61, the final decision of the Commission in this proceeding shall be issued by October 31, 2006.

By the Commission.

**Bryant L. VanBrakle**,  
Secretary.

**SCHEDULE A.—LICENSEES IN THE UNITED STATES**

Organization No.	Name
004278 .....	Cambell & Gardiner, Inc.
008727 .....	Ken Lehat & Associates, Inc.
015494 .....	Ocean Transportation Services, LLC.
011405 .....	Interfreight, Inc.
016391 .....	Caribbean American Shipping Corp.
008751 .....	Ford International Forwarding, Inc.
016817 .....	Independence Shipping Lines, Ltd.
017387 .....	S & B International Freight Forwarders, Inc.

[FR Doc. E6-3789 Filed 3-15-06; 8:45 am]

BILLING CODE 6730-01-P

**FEDERAL RESERVE SYSTEM**

**Change in Bank Control Notices; Acquisition of Shares of Bank or Bank Holding Companies**

The notificants listed below have applied under the Change in Bank

Control Act (12 U.S.C. 1817(j)) and § 225.41 of the Board's Regulation Y (12 CFR 225.41) to acquire a bank or bank holding company. The factors that are considered in acting on the notices are set forth in paragraph 7 of the Act (12 U.S.C. 1817(j)(7)).

The notices are available for immediate inspection at the Federal Reserve Bank indicated. The notices also will be available for inspection at the office of the Board of Governors. Interested persons may express their views in writing to the Reserve Bank indicated for that notice or to the offices of the Board of Governors. Comments must be received not later than March 31, 2006.

**A. Federal Reserve Bank of Atlanta**  
(Andre Anderson, Vice President) 1000 Peachtree Street, N.E., Atlanta, Georgia 30303:

1. *W.C. Martin, Jr.; Jean Wood Martin; Donald Wayne Sanders; Mary Martin Noland; Donald Martin Sanders; Rebecca Martin Sanders; William Matthew Sanders*, all of Aliceville, Alabama; *Alice Susan Martin*, Chattanooga, Tennessee; *Milton Barrett Noland*, Carrollton, Alabama; and *Karrie Noland Beasley*, Tuscaloosa, Alabama, to retain voting shares of First National Bancshares of Central Alabama, Inc., and thereby indirectly retain voting shares of First National Bank of Central Alabama, both of Aliceville, Alabama.

Board of Governors of the Federal Reserve System, March 13, 2006.

**Robert deV. Frierson**,  
*Deputy Secretary of the Board.*  
[FR Doc. E6-3811 Filed 3-15-06; 8:45 am]  
BILLING CODE 6210-01-S

**DEPARTMENT OF HEALTH AND HUMAN SERVICES**

**National Toxicology Program (NTP), NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM); Notice of Availability of a Revised List of Recommended Reference Substances for Validation of In Vitro Estrogen and Androgen Receptor Binding and Transcriptional Activation Assays: Request for Comments and Submission of In Vivo and In Vitro Data**

**AGENCY:** National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH).

**ACTION:** Request for Comments and Submission of Data.

**SUMMARY:** The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative

Toxicological Methods (NICEATM) announces the availability of an addendum to the report entitled, "Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Evaluation of *In Vitro* Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays" [NIH Publication 03-4503]. The addendum describes the rationale for proposed revisions to the original list of recommended reference substances for validation of *in vitro* estrogen receptor (ER) and androgen receptor (AR) binding and transcriptional activation (TA) assays. The original list was made publicly available in June 2003 (**Federal Register**, Vol. 68, No. 106, pp. 33171-33172, June 3, 2003). NICEATM requests public comments on the substances proposed as substitutes for six of the 78 substances in the original list. Data are also requested from *in vitro* and *in vivo* studies evaluating the estrogenic and androgenic activity of the 78 substances in the revised list of reference substances.

**DATES:** Comments and data submissions should be received by May 1, 2006.

**ADDRESSES:** Correspondence should be sent by mail, fax, or e-mail to Dr. William S. Stokes, NICEATM Director, NIEHS, P. O. Box 12233, MD EC-17, Research Triangle Park, NC, 27709, (phone) 919-541-2384, (fax) 919-541-0947, (e-mail) [niceatm@niehs.nih.gov](mailto:niceatm@niehs.nih.gov).

**SUPPLEMENTARY INFORMATION:**

**Background**

In April 2000, the Environmental Protection Agency (EPA) asked ICCVAM to evaluate the validation status of *in vitro* ER and AR binding and TA assays that were proposed as possible components of the EPA Endocrine Disruptor Screening Program Tier 1 screening battery. ICCVAM agreed to evaluate these test methods based on their potential interagency applicability and public health significance. NICEATM, which administers and provides scientific support for ICCVAM, subsequently compiled available data and information on *in vitro* ER and AR binding and TA assays in four draft Background Review Documents (BRDs) (available at <http://iccvam.niehs.nih.gov/methods/endocrine.htm>).

In collaboration with the ICCVAM Endocrine Disruptor Working Group, NICEATM organized an independent scientific evaluation of the validation status of the four types of *in vitro* endocrine disruptor screening test

methods on May 20–21, 2002, in Research Triangle Park, NC (**Federal Register**, Vol. 66, No. 57, pp. 16278–16279, March 23, 2001 and **Federal Register**, Vol. 66, No. 67, pp. 16415–16416, April 5, 2002) (available at <http://iccvam.niehs.nih.gov/methods/endocrine.htm>).

The final BRDs and the ICCVAM Test Method Evaluation Report, which includes the expert panel report, public comments, and other relevant documents, were published in May 2003 and announced in a **Federal Register** notice (Vol. 68, No. 106, pp. 33171–33172, June 3, 2003) (available at <http://iccvam.niehs.nih.gov/methods/endocrine.htm>).

NICEATM recently reviewed the commercial availability and cost for the 78 substances recommended by ICCVAM for use in *in vitro* ER and AR binding and TA validation studies. A minimum of 44 substances are recommended for AR binding and TA assays, while a minimum of 53 substances are recommended for ER binding and TA assays. This review indicated that three substances [anastrozole, CGS 18320B, fadrozole] are not commercially available, one substance has restricted commercial availability [ICI 182,780] and six others [actinomycin D, hydroxyflutamide, 4-hydroxytamoxifen, methyltrienolone, 12-O-tetradecanoylphorbol-13-acetate, zearalenone] have costs that are considered excessive. ICCVAM proposes replacing the four substances that are not commercially available or have restricted availability with ones having similar ER and AR activity profiles [4-hydroxyandrostenedione, chrysin, dicofol, raloxifene HCl]. Suitable replacements (19-nortestosterone and resveratrol) were identified for methyltrienolone and zearalenone, respectively, for two of the expensive substances. NICEATM would also prefer to replace four of the highly priced substances [actinomycin D, hydroxyflutamide, 4-hydroxytamoxifen, 12-O-tetradecanoylphorbol-13-acetate], but has been unable to identify suitable replacements because of their unique activity profiles and/or chemical/physical properties. The revised list of 78 substances and a discussion about the proposed revisions are included and discussed in the "Addendum to the ICCVAM Evaluation of In Vitro Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays." (available at <http://iccvam.niehs.nih.gov> see "Test Method Evaluations") or by contacting NICEATM (see **ADDRESSES** above.) ICCVAM will finalize this list

after considering any public comments received and forward it to U.S. Federal agencies for their information and consideration.

#### Request for Comments and Request for Data

NICEATM requests public comments on the four substances (listed above) proposed as replacements for substances on the list that are not readily commercially available. NICEATM also requests public comments on the proposed replacements for the two expensive substances for which replacements have been identified, and suggestions for replacements for the four expensive substances that remain on the recommended list.

In order to update the reference substance database, NICEATM request data from completed *in vitro* studies using or evaluating ER and AR binding and/or TA assays, and information about ongoing or planned studies using or evaluating these test methods. NICEATM also requests the submission of data from animal studies that have evaluated the endocrine activity of chemicals using, for example, the uterotrophic, Hershberger, intact male, or male/female pubertal assays. NICEATM is especially interested in receiving additional data or information on any of the 78 substances included in the reference list. NICEATM previously requested data from completed studies using or evaluating ER and AR binding and/or TA assays, and information about ongoing or planned *in vitro* or *in vivo* studies using or evaluating these test methods (**Federal Register**, Vol. 66, No. 57, pp. 16278–16279, March 23, 2001). Submitted data will be used to update and supplement the existing NICEATM database; the current database can be accessed in the ICCVAM Test Method Evaluation Report [NIH Publication No. 03–4503] and the four final BRDs on ER and AR binding and TA assays [NIH Publication No. 03–4504, 03–4505, 03–4506, and 03–4507] (available at <http://iccvam.niehs.nih.gov/methods/endocrine.htm>).

When submitting chemical and protocol information/test data, please reference this **Federal Register** notice and provide appropriate contact information (name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization, as applicable).

NICEATM prefers data to be submitted as copies of pages from study notebooks and/or study reports, if available. Raw data and analyses available in electronic format may also be submitted. If data are published in the peer-reviewed literature, citations

should be provided. Each submission for a chemical should preferably include the following information, as appropriate:

- Common and trade name
- Chemical Abstracts Service Registry Number (CASRN)
- Chemical class
- Product class
- Commercial source
- *In vitro* test protocol used
- *In vitro* test results
- *In vivo* test protocol used
- *In vivo* test results
- The extent to which the study complied with national or international Good Laboratory Practice (GLP) guidelines
- Date and testing organization

#### Background Information on ICCVAM and NICEATM

ICCVAM is an interagency committee composed of representatives from 15 Federal regulatory and research agencies that use or generate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability and promotes the scientific validation and regulatory acceptance of toxicological test methods that more accurately assess the safety and hazards of chemicals and products and that refine, reduce, or replace animal use. The ICCVAM Authorization Act of 2000 (Pub. L. 106–545) establishes ICCVAM as a permanent interagency committee of the NIEHS under the NICEATM. NICEATM administers the ICCVAM and provides scientific and operational support for ICCVAM-related activities. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of Federal agencies. Additional information about ICCVAM and NICEATM can be found at the following Web site: <http://www.iccvam.niehs.nih.gov>.

Dated: March 7, 2006.

**Samuel H. Wilson,**

*Deputy Director, National Institute of Environmental Health Sciences and National Toxicology Program.*

[FR Doc. E6–3763 Filed 3–15–06; 8:45 am]

**BILLING CODE 4140–01–P**



## **Appendix C-2**

### **71FR56997 Notice of Availability of a Revised List of Recommended Reference Substances for Validation of In Vitro Estrogen and Androgen Receptor Binding and Transcriptional Activation Assays**

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**Name of Committee:** Center for Scientific Review Special Emphasis Panel; Assays and Detectors.

**Date:** October 25–26, 2006.

**Time:** 6 p.m. to 7 p.m.

**Agenda:** To review and evaluate grant applications.

**Place:** Clarion Hotel Bethesda Park, 8400 Wisconsin Avenue, Bethesda, MD 20814.

**Contact Person:** Geoffrey White, PhD, Scientific Review Administrator, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 5148, MSC 7849, Bethesda, MD 20892, (301) 435-1735, [white@csr.nih.gov](mailto:white@csr.nih.gov).

(Catalogue of Federal Domestic Assistance Program Nos. 93.306, Comparative Medicine; 93.333, Clinical Research; 93.306, 93.333, 93.337, 93.393–93.396, 93.837–93.844, 93.846–93.878, 93.892, 93.983, National Institutes of Health, HHS)

**Dated:** September 19, 2006.

**Anna Snouffer,**

*Acting Director, Office of Federal Advisory Committee Policy.*

[FR Doc. 06-8331 Filed 9-27-06; 8:45 am]

**BILLING CODE 4140-01-M**

## DEPARTMENT OF HEALTH AND HUMAN SERVICES

### National Institutes of Health

#### National Toxicology Program (NTP), NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM); Notice of Availability of a Revised List of Recommended Reference Substances for Validation of *In Vitro* Estrogen and Androgen Receptor Binding and Transcriptional Activation Assays

**AGENCY:** National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH).

**ACTION:** Notice of the availability of a revised list of recommended reference substances.

**SUMMARY:** NICEATM announces the availability of an addendum to the report, "Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Evaluation of *In Vitro* Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays" [NIH Publication 03-4503]. The addendum describes the rationale for revisions to the original list of recommended reference substances for validation of *in vitro* estrogen receptor (ER) and androgen receptor (AR) binding and transcriptional activation (TA) assays.

#### SUPPLEMENTARY INFORMATION:

#### Background

In April 2000, the Environmental Protection Agency (EPA) asked ICCVAM to evaluate the validation status of *in vitro* ER and AR binding and TA assays that were proposed as possible components of the EPA Endocrine Disruptor Screening Program Tier 1 screening battery. ICCVAM agreed to evaluate these test methods based on their potential interagency applicability and public health significance. NICEATM subsequently compiled available data and information on *in vitro* ER and AR binding and TA assays in four draft Background Review Documents (BRDs) (available at <http://iccvam.niehs.nih.gov/methods/endocrine.htm>).

In collaboration with the ICCVAM Endocrine Disruptor Working Group, NICEATM organized an independent scientific evaluation of the validation status of the four types of *in vitro* endocrine disruptor screening test methods on May 20–21, 2002, in Research Triangle Park, NC (**Federal Register**, Vol. 66, No. 57, pp. 16278–16279, March 23, 2001 and **Federal Register**, Vol. 66, No. 67, pp. 16415–16416, April 5, 2002, available at <http://iccvam.niehs.nih.gov/methods/endocrine.htm>).

The final BRDs and the ICCVAM Test Method Evaluation Report, which includes the expert panel report, public comments, and other relevant documents, were published in May 2003 and announced in the **Federal Register** notice (Vol. 68, No. 106, pp. 33171–33172, June 3, 2003, available at <http://iccvam.niehs.nih.gov/methods/endocrine.htm>).

NICEATM recently reviewed the commercial availability and cost for the 78 substances recommended by ICCVAM for use in *in vitro* ER and AR binding and TA validation studies. A minimum of 44 substances are recommended for AR binding and TA assays, while a minimum of 53 substances are recommended for ER binding and TA assays. This review indicated that three substances (anastrozole, CGS 18320B, and fadrozole) are not commercially available, one substance has restricted commercial availability (ICI 182,780) and six others (actinomycin D, hydroxyflutamide, 4-hydroxytamoxifen, methyltrienolone, 12-O-tetradecanoylphorbol-13-acetate, zearalenone) have costs that are considered excessive. ICCVAM has replaced the four substances, which are not commercially available or have restricted availability, with ones having similar ER and AR activity profiles (4-

hydroxyandrostenedione, chrysin, dicofol, raloxifene HCl), 19-nortestosterone and resveratrol were identified as replacements for two of the expensive substances, methyltrienolone and zearalenone respectively. NICEATM sought to replace four of the highly priced substances (actinomycin D, hydroxyflutamide, 4-hydroxytamoxifen, 12-O-tetradecanoylphorbol-13-acetate), but was unable to identify suitable replacements because of their unique activity profiles and/or chemical/physical properties. The proposed revisions were made available for public comment in March 2006 (**Federal Register**, Vol. 71, No. 51, pp. 13597–13598, March 16, 2006) and no comments were received. The final revised list of 78 reference substances recommended for validation of *in vitro* ER and AR binding and TA validation studies and a discussion about the revisions are now available in the document, "Addendum to the ICCVAM Evaluation of *In Vitro* Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays." The addendum is available on the ICCVAM/NICEATM Web site at <http://iccvam.niehs.nih.gov> see "Test Method Evaluations" or by contacting NICEATM (requests should be sent by mail, fax, or e-mail to Dr. William S. Stokes, NICEATM Director, NIEHS, P. O. Box 12233, MD EC-17, Research Triangle Park, NC, 27709, (phone) 919-541-2384, (fax) 919-541-0947, (e-mail) [niceatm@niehs.nih.gov](mailto:niceatm@niehs.nih.gov)).

#### Background Information on ICCVAM and NICEATM

ICCVAM is an interagency committee composed of representatives from 15 Federal regulatory and research agencies that use or generate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability and promotes the scientific validation and regulatory acceptance of toxicological test methods that more accurately assess the safety and hazards of chemicals and products and that refine, reduce, or replace animal use. The ICCVAM Authorization Act of 2000 (42 U.S.C. 285) establishes ICCVAM as a permanent interagency committee of the NIEHS under the NICEATM. NICEATM administers the ICCVAM and provides scientific and operational support for ICCVAM-related activities. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of Federal agencies. Additional information about ICCVAM and

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NICEATM can be found at the following Web site: <http://www.iccvam.niehs.nih.gov>.

Dated: September 18, 2006.

**Samuel H. Wilson,**

*Deputy Director, National Institute of Environmental Health Sciences and National Toxicology Program.*

[FR Doc. E6-15972 Filed 9-27-06; 8:45 am]

BILLING CODE 4140-01-P

## DEPARTMENT OF HOMELAND SECURITY

### Coast Guard

[USCG 2006-25080]

#### Medical and Physical Evaluation Guidelines for Merchant Mariner Credentials

**ACTION:** Notice of availability; request for comments.

**SUMMARY:** The Coast Guard announces the availability of, and seeks public comment on, a draft Navigation and Vessel Inspection Circular (NVIC) to replace the existing NVIC 2-98, "Physical Evaluation Guidelines for Merchant Mariner's Documents and Licenses." The new proposed NVIC is entitled "Medical and Physical Evaluation Guidelines for Merchant Mariner Credentials." It will be officially numbered if and when it becomes effective. The contents of this NVIC were developed from recommendations and input provided by the Merchant Marine Personnel Advisory Committee (MERPAC) and experienced maritime community medical practitioners. A copy of the proposed NVIC has been posted to the public docket for this notice, and it is available as described under **ADDRESSES**. **DATES:** Comments and related material must reach the Docket Management Facility on or before November 27, 2006.

**ADDRESSES:** The proposed NVIC is available on the Internet at <http://dms.dot.gov>, under this docket number [USCG 2006-25080]. It is also available from Mr. Mark Gould, Maritime Personnel Qualifications Division, Office of Operating and Environmental Standards, Commandant (G-PSO-1), U.S. Coast Guard Headquarters, telephone 202-372-1409, or e-mail address: [Mark.C.Gould@uscg.mil](mailto:Mark.C.Gould@uscg.mil).

The Coast Guard encourages you to submit comments. The most helpful comments will include the specific section of the proposed NVIC to which each comment applies, as well as the reason for each comment. Comments

should be identified by USCG docket number USCG-2006-25080. Please include your name and address with your comments and submit using ONE of the following methods:

(1) *Web site:* <http://dms.dot.gov>.

(2) *Mail:* Docket Management Facility, U.S. Department of Transportation, 400 Seventh Street, SW., Room PL-401, Washington, DC 20590-0001.

(3) *Fax:* 202-493-2251.

(4) *Delivery:* Room PL-401 on the Plaza level of the Nassif Building, 400 Seventh Street, SW., Washington, DC, between 9 a.m. and 5 p.m., Monday through Friday, except Federal holidays. The telephone number is 202-366-9329.

(5) *Federal eRulemaking Portal:* <http://www.regulations.gov>. Follow the instructions on the Web site.

The Docket Management Facility maintains the public docket for this notice. Comments and related material received from the public, as well as documents mentioned in this notice (including the proposed NVIC), will become part of this docket and will be available for inspection or copying at room PL-401 on the Plaza level of the Nassif Building, 400 Seventh Street, SW., Washington, DC 20590-0001, between 9 a.m. and 5 p.m., Monday through Friday, except Federal holidays. Copies of the docket may also be viewed on the internet at: <http://dms.dot.gov> and <http://www.regulations.gov>.

If you mail or deliver your comments and material, they must be on 8½-by-11-inch paper, and the quality of the copy should be clear enough for copying and scanning. If you mail your comments and material and would like to know whether the Docket Management Facility received them, please enclose a stamped, self-addressed postcard or envelope. The Coast Guard will consider all comments and material received during the 60-day comment period.

**FOR FURTHER INFORMATION CONTACT:** For questions on this notice or on the proposed NVIC, e-mail or call Mr. Gould where indicated under **ADDRESSES**. For questions on viewing or submitting material to the docket, call Ms. Renee V. Wright, Program Manager, Docket Management System, U.S. Department of Transportation, Room Plaza 401, 400 Seventh Street, SW., Washington, DC 20590-0001; telephone (202) 493-0402.

#### SUPPLEMENTARY INFORMATION:

##### What action is the Coast Guard taking?

The proposed NVIC contains revised guidelines for evaluating the physical and medical conditions of applicants for merchant mariner's documents (MMD),

licenses, certificates of registry and STCW endorsements, collectively referred to as "credential(s)." The purpose of the proposed NVIC is to replace the existing NVIC 2-98. It also provides guidance for evaluating the physical and medical conditions of applicants for merchant mariner credentials (MMCs), if and when the Coast Guard begins issuing MMCs as proposed in 71 FR 29462, "Consolidation of Merchant Mariner Qualification Credentials."

##### Why is the Coast Guard taking this action?

The International Convention on Standards of Training, Certification and Watchkeeping for Seafarers, 1978, as amended (STCW) requires each party to establish standards of medical fitness for seafarers. Title 46 United States Code, Subtitle II, Part E, and Title 46 Code of Federal Regulations (CFR) subpart B require that mariners be physically able to perform their duties, using terms such as "general physical condition," "good health" and "of sound health." Title 46 CFR parts 401 and 402 contain special requirements for registration as a Great Lakes Pilot, including the requirement to "pass a physical examination given by a licensed medical doctor." None of these references contain specific standards, with the exception of visual acuity and color vision, for determining if mariners are physically and medically qualified.

The lack of specificity in the above statutes and regulations has led to confusion and unnecessary delays in processing credential applications as well as inconsistent evaluations by medical practitioners conducting examinations of credential applicants. Moreover, it has caused confusion on the part of Coast Guard personnel charged with determining whether a credential should be issued. The proposed NVIC provides the specificity that the above statutes and regulations lack. It details the specific medical and physical conditions that are potentially disqualifying, and the data recommended for evaluation of each of these conditions. This is expected to reduce the inconsistency and subjectivity of the medical evaluation process and eliminate the guesswork that mariners may currently encounter as to what specific physical and medical information is needed to process their applications.

In addition, there are public safety risks associated with some medical and physical conditions, particularly when these conditions may result in the sudden incapacitation of mariners on vessels. These conditions can be the