

Do Not Cite, Quote, or Distribute

1	Table of Contents	
2	3.0 Substances Used for the Validation of the BG1Luc ER TA	2
3	3.1 Development of the List of 78 ICCVAM Recommended Test Substances	2
4	3.2 Substances Used to Evaluate Test Method Accuracy	9
5	3.3 Substances Used to Evaluate Concordance with Other Accepted Methods	14
6	3.3.1 Substances Used to Evaluate ER TA Assay Concordance with the CERI-STTA Test	
7	Method 14	
8	3.3.2 Substances Used to Evaluate EA TA Assay Concordance with <i>In Vitro</i> ER Binding	
9	Test Methods	15
10	3.3.3 Substances Used to Evaluate EA TA Assay Concordance with the <i>In Vivo</i> Uterotrophic	
11	Bioassay	16
12	3.4 Substances Tested in Each Phase of Validation	17
13	3.5 Substances Used for Intralaboratory Reproducibility	29
14	3.6 Substances Used to Assess Interlaboratory Reproducibility	29
15	3.7 Chemical Classes Represented by the List of Substances	29
16	3.8 Product Classes Represented by the List of Substances	30
17	3.9 Test Substance Procurement, Coding, and Distribution	31
18		
19		

Do Not Cite, Quote, or Distribute

19

20 **3.0 Substances Used for the Validation of the BG1Luc ER TA**21 **3.1 Development of the List of 78 ICCVAM Recommended Test Substances**

22 ICCVAM previously compiled a list of 78 substances that are recommended for use in validation studies
 23 for *in vitro* ER and androgen receptor (AR) binding and TA test methods (ICCVAM 2003, 2006). The
 24 purpose of this list is to ensure that the usefulness and limitations of *in vitro* ER and AR binding and TA
 25 assays can be adequately characterized across a broad range of chemical classes and responses. These
 26 substances were selected based on information contained in the ICCVAM BRDs for AR and ER binding
 27 and TA test methods (ICCVAM 2002d, 2002b, 2002a, 2002c), as well as information obtained from
 28 publications reviewed or published after completion of the ICCVAM BRDs (**Annex N**). Factors and
 29 criteria considered in compiling the list included:

- 30 • The availability of published or submitted data demonstrating reproducible positive or
 31 negative responses in multiple studies and/or test methods
- 32 • The extent to which these substances covered the range of responses (negative, weakly
 33 positive to strongly positive)
- 34 • Representative distribution of the proposed substances among chemical and product classes.
- 35 • To better evaluate test method specificity, approximately 25% of the total number of
 36 substances should be negative for the endpoint being measured.
- 37 • Substances that might interfere with transcriptional activation by altering metabolic
 38 pathways, such as RNA and protein synthesis, should be included.

39 The list of 78 ICCVAM-recommended substances used in the BG1Luc ER TA validation study is
 40 provided in **Table 3-1**. Physicochemical properties, including chemical structures, for each of the
 41 recommended substances are provided in **Annex I**.

42 **Table 3-1 List of Reference Substances Tested for ER TA Activity**

Substance	CASRN	MESH Chemical Class ^a	Product Class ^b	Purity (%)	Manufacturer
12- <i>O</i> -Tetradecanoylphorbol-13-acetate	16561-29-8	Hydrocarbon (Cyclic)	Laboratory Chemical	>99.5	LC Laboratories, Inc.
17 β -Estradiol	50-28-2	Steroid	Pharmaceutical, Veterinary Agent	98.0	Sigma-Aldrich Corporation

Do Not Cite, Quote, or Distribute

Substance	CASRN	MESH Chemical Class ^a	Product Class ^b	Purity (%)	Manufacturer
17 β -Trenbolone	10161-33-8	Steroid	Pharmaceutical	96.6	Spectrum Chemicals & Laboratory Products
17 α -Estradiol	57-91-0	Steroid	Pharmaceutical, Veterinary Agent	99.5	Sigma-Aldrich Corporation
17 α -Ethinyl estradiol	57-63-6	Steroid	Pharmaceutical, Veterinary Agent	\geq 98.0	Sigma-Aldrich Corporation
19-Nortestosterone	434-22-0	Steroid	Pharmaceutical, Veterinary Agent	98.0	TRC
2- <i>sec</i> -Butylphenol	89-72-5	Phenol	Chemical Intermediate, Pesticide Intermediate	98.0	Sigma-Aldrich Corporation
2,4,5-Trichlorophenoxyacetic acid	93-76-5	Carboxylic Acid	Herbicide	99.3	Sigma-Aldrich Corporation
4-Androstenedione	63-05-8	Steroid	Pharmaceutical	98.6	Sigma-Aldrich Corporation/ Hiyoshi-International Laboratory USA
4-Cumylphenol	599-64-4	Phenol	Chemical Intermediate	99.9	Sigma-Aldrich Corporation
4-Hydroxy-tamoxifen	68047-06-3	Hydrocarbon (Cyclic)	Pharmaceutical	99.5	Sigma-Aldrich Corporation
4-Hydroxyandrostenedione	566-48-3	Steroid	Pharmaceutical	99.6	Sigma-Aldrich Corporation
4- <i>tert</i> -Octylphenol	140-66-9	Phenol	Chemical Intermediate, Pharmaceutical Intermediate	99.3	ChemService, Inc.
5 α -Dihydrotestosterone	521-18-6	Steroid	Pharmaceutical	\geq 97.5	Sigma-Aldrich Corporation

Do Not Cite, Quote, or Distribute

Substance	CASRN	MESH Chemical Class ^a	Product Class ^b	Purity (%)	Manufacturer
Actinomycin D	50-76-0	Heterocyclic Compound, Polycyclic Compound	Laboratory Chemical, Pharmaceutical, Veterinary Agent	99.7	USB Corp
Ammonium perchlorate	7790-98-9	Amine, Onium Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical	100.0	Sigma-Aldrich Corporation
Apigenin	520-36-5	Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate	>99.0	Sigma-Aldrich Corporation
Apomorphine	58-00-4	Heterocyclic Compound	Pharmaceutical, Veterinary Agent	99.8	Sigma-Aldrich Corporation
Atrazine	1912-24-9	Heterocyclic Compound	Herbicide	98.0	ChemService, Inc.
Bicalutamide	90357-06-5	Amide	Pharmaceutical	>99.5	LKT Laboratories, Inc.
Bisphenol A	80-05-7	Phenol	Chemical Intermediate, Flame Retardant, Fungicide	97.0	Sigma-Aldrich Corporation
Bisphenol B	77-40-7	Phenol	Chemical Intermediate, Flame Retardant, Fungicide	97.4	City Chemical LLC
Butylbenzyl phthalate	85-68-7	Carboxylic Acid, Ester, Phthalic Acid	Plasticizer, Industrial Chemical	98.0	Sigma-Aldrich Corporation
Chrysin	480-40-0	Flavonoid, Heterocyclic Compound	Natural Product	99.8	Sigma-Aldrich Corporation
Clomiphene citrate	50-41-9	Amine, Carboxylic Acid, Heterocyclic Compound	Pharmaceutical	100.0	Sigma-Aldrich Corporation

Do Not Cite, Quote, or Distribute

Substance	CASRN	MESH Chemical Class ^a	Product Class ^b	Purity (%)	Manufacturer
Corticosterone	50-22-6	Steroid	Pharmaceutical	99.0	Sigma-Aldrich Corporation
Coumestrol	479-13-0	Heterocyclic Compound	Natural Product	98.0	BIOMOL International, Inc.
Cyclohexamide	66-81-9	Heterocyclic Compound	Fungicide, Pharmaceutical, Veterinary Agent	99.0	Sigma-Aldrich Corporation
Cyproterone acetate	427-51-0	Steroid	Pharmaceutical	99.6	Sigma-Aldrich Corporation
Daidzein	486-66-8	Flavonoid, Heterocyclic Compound	Natural Product	≥97.5	Alfa Aesar GmbH
Dexamethasone	50-02-2	Steroid	Pharmaceutical, Veterinary Agent	99.0	Sigma-Aldrich Corporation
Di- <i>n</i> -butyl phthalate	84-74-2	Ester, Phthalic Acid	Cosmetic Ingredient, Industrial Chemical, Plasticizer	≥98.0	City Chemical LLC
Dibenzo[<i>a,h</i>] Anthracene	53-70-3	Polycyclic Compound	Laboratory Chemical, Natural Product	99.9	Supelco Analytical
Dicofol	115-32-2	Hydrocarbon (Cyclic), Hydrocarbon (Halogenated)	Pesticide	98.0	ChemService, Inc.
Diethylhexyl phthalate	117-81-7	Phthalic Acid	Pesticide Intermediate, Plasticizer	98.0	Alfa Aesar GmbH
Diethylstilbestrol	56-53-1	Hydrocarbon (Cyclic)	Pharmaceutical, Veterinary Agent	≥99.0	Sigma-Aldrich Corporation
Estrone	53-16-7	Steroid	Pharmaceutical, Veterinary Agent	99.0	Sigma-Aldrich Corporation
Ethyl paraben	120-47-8	Carboxylic Acid, Phenol	Pharmaceutical, Preservative	99.0	Sigma-Aldrich Corporation

Do Not Cite, Quote, or Distribute

Substance	CASRN	MESH Chemical Class^a	Product Class^b	Purity (%)	Manufacturer
Fenarimol	60168-88-9	Heterocyclic Compound, Pyrimidine	Fungicide	99.5	ChemService, Inc.
Finasteride	98319-26-7	Steroid	Pharmaceutical	>99.0	Sigma-Aldrich Corporation
Flavone	525-82-6	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical	99.7	Sigma-Aldrich Corporation
Fluoranthene	206-44-0	Polycyclic Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical Intermediate	99.6	Sigma-Aldrich Corporation
Fluoxymestrone	76-43-7	Steroid	Pharmaceutical	>99.0	Sigma-Aldrich Corporation
Flutamide	13311-84-7	Amide	Pharmaceutical, Veterinary Agent	100.0	Sigma-Aldrich Corporation
Genistein	446-72-0	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical	98.8	Sigma-Aldrich Corporation
Haloperidol	52-86-8	Ketone	Pharmaceutical, Veterinary Agent	>99.0	Sigma-Aldrich Corporation
Hydroxy flutamide	52806-53-8	Amide	Pharmaceutical	99.4	LKT Laboratories, Inc.
Kaempferol	520-18-3	Flavonoid, Heterocyclic Compound	Natural Product	99.0	Indofine Chemical Company, Inc.
Kepone	143-50-0	Hydrocarbon (Halogenated)	Pesticide	>99.9	Supelco Analytical
Ketoconazole	65277-42-1	Heterocyclic Compound	Pharmaceutical	>99.0	Sigma-Aldrich Corporation
L-Thyroxine	51-48-9	Amino Acid	Pharmaceutical, Veterinary Agent	98.0	Sigma-Aldrich Corporation
Linuron	330-55-2	Urea	Herbicide	99.5	ChemService, Inc

Do Not Cite, Quote, or Distribute

Substance	CASRN	MESH Chemical Class ^a	Product Class ^b	Purity (%)	Manufacturer
Medroxyprogesterone acetate	71-58-9	Steroid	Pharmaceutical	99.0	Sigma-Aldrich Corporation
<i>meso</i> -Hexestrol	84-16-2	Steroid	Pharmaceutical, Veterinary Agent	99.3	City Chemical LLC
Methyl testosterone	58-18-4	Steroid	Pharmaceutical, Veterinary Agent	99.0	Sigma-Aldrich Corporation
Mifepristone	84371-65-3	Steroid	Pharmaceutical	99.1	Sigma-Aldrich Corporation
Morin	480-16-0	Flavonoid, Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate	95.3	TCI America
Nilutamide	63612-50-0	Heterocyclic Compound, Imidazole	Pharmaceutical	100.0	Sigma-Aldrich Corporation
Norethynodrel	68-23-5	Steroid	Pharmaceutical	≥95.0	Research Plus, Inc.
<i>o,p'</i> -DDT	789-02-6	Hydrocarbon (Halogenated)	Pesticide	98.9	ChemService, Inc.
Oxazepam	604-75-1	Heterocyclic Compound	Pharmaceutical, Veterinary Agent	99.5	Sigma-Aldrich Corporation
<i>p-n</i> -Nonylphenol	104-40-5	Phenol	Chemical Intermediate	99.6	Alfa Aesar GmbH
<i>p,p'</i> -Methoxychlor	72-43-5	Hydrocarbon (Halogenated)	Pesticide, Veterinary Agent	99.1	ChemService, Inc.
<i>p,p'</i> -DDE	72-55-9	Hydrocarbon (Halogenated)	Pesticide Intermediate	99.0	Sigma-Aldrich Corporation
Phenobarbital	50-06-6	Heterocyclic Compound, Pyrimidine	Pharmaceutical, Veterinary Agent	100.0	Spectrum Chemical Mfg. Corp
Phenolphthalin	81-90-3	Carboxylic Acid, Phenol	Dye, Laboratory Chemical	95.0	Sigma-Aldrich Corporation
Pimozide	2062-78-4	Heterocyclic Compound	Pharmaceutical	>99.0	Sigma-Aldrich Corporation

Do Not Cite, Quote, or Distribute

Substance	CASRN	MESH Chemical Class ^a	Product Class ^b	Purity (%)	Manufacturer
Procymidone	32809-16-8	Polycyclic Compound	Fungicide	99.0	ChemService, Inc.
Progesterone	57-83-0	Steroid	Pharmaceutical, Veterinary Agent	≥99.0	Sigma-Aldrich Corporation
Propylthiouracil	51-52-5	Heterocyclic Compound, Pyrimidine	Pharmaceutical, Veterinary Agent	100.0	Sigma-Aldrich Corporation
Raloxifene HCl	82640-04-8	Hydrocarbon (Cyclic)	Pharmaceutical	100.0	Sigma-Aldrich Corporation
Reserpine	50-55-5	Heterocyclic Compound, Indole	Pharmaceutical, Veterinary Agent	98.0	Sigma-Aldrich Corporation
Resveratrol	501-36-0	Hydrocarbon (Cyclic)	Natural Product	≥99.0	Sigma-Aldrich Corporation
Sodium azide	26628-22-8	Azide, Salt (Inorganic)	Chemical Intermediate, Fungicide, Herbicide	99.7	Sigma-Aldrich Corporation
Spironolactone	52-01-7	Lactone, Steroid	Pharmaceutical	99.7	Sigma-Aldrich Corporation
Tamoxifen	10540-29-1	Hydrocarbon (Cyclic)	Pharmaceutical	≥99.0	Sigma-Aldrich Corporation
Testosterone	58-22-0	Steroid	Pharmaceutical, Veterinary Agent	>99.0	Sigma-Aldrich Corporation
Vinclozolin	50471-44-8	Heterocyclic Compound	Fungicide	99.5	ChemService, Inc.

43 Abbreviations: CASRN = Chemical Abstracts Service Registry Number; MeSH = U.S. National Library of Medicine's Medical
 44 Subject Headings

45 ^aSubstances were assigned into one or more chemical classes using the U.S. National Library of Medicine's Medical Subject
 46 Headings (MeSH), an internationally recognized standardized classification scheme (available at: <http://www.nlm.nih.gov/mesh>).

47 ^bSubstances were assigned into one or more product classes using the U.S. National Library of Medicine's Hazardous Substances
 48 Database (available at: <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>)

49

50 The following sections describe the subsets of this list that were used to evaluate BG1Luc ER TA
 51 accuracy and reproducibility, as well as the rationale for selection of each subset. The data and rationale
 52 used to establish a reference classification for each of the 78 substances are also discussed.

Do Not Cite, Quote, or Distribute

53 3.2 Substances Used to Evaluate Test Method Accuracy

54 Accuracy is the degree of closeness of agreement between a test method result and an accepted reference
55 value; the extent to which a test method obtains the “correct” answer. The ICCVAM list of 78
56 recommended reference substances was developed to assess test method performance of four different
57 assays (ER TA and AR TA agonist and antagonist assays), each with its own unique set of “correct”
58 classifications for these substances. However, this validation study is focused only on the ability of the
59 BG1Luc ER TA to detect substances with *in vitro* ER TA agonist or antagonist activity. Therefore, each
60 of the 78 reference substances was assigned a classification specific for ER TA agonist and ER TA
61 antagonist activity based on a preponderance of evidence found in a review of the scientific literature.
62 NICEATM conducted a broad literature search using on-line sources including SCOPUS, PUBMED, and
63 Web of Science. Publically available information from U.S government agencies and the OECD was also
64 considered. This search strategy yielded 103 publications with relevant ER TA data. The following
65 information was extracted from each reference, and is provided in **Annex N**:

- 66 • Name, source, and purity of the substance being tested.
- 67 • Characteristics of cell line (e.g., name of cell line, tissue of origin)
- 68 • Reporter gene construct (e.g., ER source, reporter vector, endpoint measured, whether cell
69 toxicity measurements were made, and transfection method [i.e., whether stable or transient])
- 70 • Assay type (i.e., agonism or antagonism)
- 71 • Any relevant quantitative information (e.g., IC₅₀, EC₅₀, ED₅₀)

72 As would be expected, there was considerable disparity in the number of ER TA references applicable to
73 each substance. Therefore, the following criteria were used to classify each reference substance with
74 respect to ER TA agonist and antagonist activity:

- 75 • A substance was classified as Positive (POS) if it was reported as positive in > 50% of
76 referenced ER TA studies.
- 77 • A substance was classified as Negative (NEG) if it was reported as negative in all referenced
78 ER TA studies (minimum of two studies were required for Negative classification).
- 79 • A substance was classified as Presumed Positive (PP) if it was positive in 50% or less of
80 referenced ER TA studies, or if it was reported positive in the single study conducted.
- 81 • A substance was classified as Presumed Negative (PN) if it was reported negative in a single
82 ER TA study.
- 83 • Substances without data were classified as PP or PN based on other available information,
84 including their known mechanism of action or their responses in other ER assays.

Do Not Cite, Quote, or Distribute

85 A summary of the literature findings for all ICCVAM Reference Substances is provided in **Table 3-2**,
 86 along with the resulting ER TA classifications for agonist and antagonist activity based on the criteria
 87 provided above. Only those substances that could be definitively classified as POS or NEG were used to
 88 assess accuracy (substances classified as PP or PN were not considered when evaluating test method
 89 accuracy), resulting in 48 unique substances used for the assessment of accuracy. Separate lists were
 90 generated for evaluating accuracy based on agonist (42 substances; 33 Positive, 9 Negative) and
 91 antagonist (25 substances; 3 Positive, 22 Negative) activity. There were 19 substances common to both
 92 reference lists. The 42 reference substances used to assess accuracy based on ER agonist activity are
 93 provided in **Table 3-3**, and the 25 reference substances used to assess accuracy based on ER antagonist
 94 activity are provided in **Table 3-4**.

95 **Table 3-2 ER Agonist and Antagonist TA Assay Reference Data Summary**

ICCVAM Reference Substance	CASRN	ER TA Agonist Activity ^a	ER TA Antagonist Activity ^b	ER Binding Activity ^c	CERI ER TA Activity ^d	Uterotrophic Activity ^e
12- <i>O</i> -tetradecanoyl-phorbol-13-acetate	16561-29-8	PN (nt)	PN (nt)	PN (nt)	nt	nt
17- β estradiol	50-28-2	POS (226/226)	PP (1/1)	POS (160/160)	POS	nt
17- α estradiol	57-91-0	POS (10/10)	PP (1/1)	POS (15/15)	POS	POS (nt/+)
17- α ethinyl estradiol	57-63-6	POS (21/21)	NEG (0/9)	POS (32/32)	POS	POS (+/+)
17 β -trenbolone	10161-33-8	PP (1/1)	PN (nt)	PN (nt)	POS	nt
19-nortestosterone*	434-22-0	POS (3/3)	PP (1/1)	PP (1/7)	nt	nt
2- <i>sec</i> -butylphenol	89-72-5	PN (0/1)	PN (nt)	POS (2/2)	NEG	nt
2,4,5-trichlorophenoxy-acetic acid	93-76-5	PP (1/3)	PP (1/2)	PP (1/3)	nt	nt
4-androstenedione	63-05-8	PP (1/1)	PN (0/1)	PP (1/5)	NEG	nt
4-cumylphenol	599-64-4	POS (4/4)	PN (nt)	POS (3/3)	POS	nt
4-hydroxy androstenedione*	566-48-3	PP (1/2)	PN (nt)	PP (nt)	nt	nt
4-hydroxytamoxifen	68047-06-3	PP (17/56)	POS (27/27)	POS (36/36)	nt	nt
4- <i>tert</i> -octylphenol	140-66-9	POS (20/23)	PN (nt)	POS (20/20)	POS	POS (nt/+)
5 α -dihydro testosterone	521-18-6	POS (15/17)	NEG (0/3)	POS (17/18)	nt	POS (nt/+)
Actinomycin D	50-76-0	PN (nt)	PN (nt)	PN (nt)	nt	nt
Ammonium perchlorate	7790-98-9	PN (nt)	PN (nt)	PN (nt)	nt	nt
Apigenin	520-36-5	POS (25/25)	NEG (0/11)	POS	POS	nt
Apomorphine	58-00-4	PN (nt)	PN (nt)	PN (nt)	nt	nt
Atrazine	1912-24-9	NEG (0/29)	PN (0/1)	PP (2/19)	NEG	nt
Bicalutamide	90357-06-5	NEG (0/5)	PN (nt)	PN (nt)	nt	nt
Bisphenol A	80-05-7	POS (64/64)	NEG (0/12)	POS (46/47)	POS	POS (+/+)
Bisphenol B	77-40-7	POS (5/5)	PN (0/1)	POS (2/2)	POS	POS (nt/+)
Butylbenzyl phthalate	85-68-7	POS (11/13)	NEG (0/3)	POS (10/19)	POS	NEG (-/-)
Chrysin*	480-40-0	POS (6/9)	NEG (0/4)	PP (2/10)	nt	nt
Clomiphene citrate	50-41-9	POS (3/4)	PP (1/1)	POS (8/8)	POS	nt
Corticosterone	50-22-6	NEG (0/5)	PP (1/3)	NEG (0/6)	NEG	nt
Coumestrol	479-13-0	POS (29/29)	NEG (0/8)	POS (38/38)	POS	nt
Cycloheximide	66-81-9	PN (nt)	PP (nt)	PN (nt)	nt	nt

Do Not Cite, Quote, or Distribute

ICCVAM Reference Substance	CASRN	ER TA Agonist Activity ^a	ER TA Antagonist Activity ^b	ER Binding Activity ^c	CERI ER TA Activity ^d	Uterotrophic Activity ^e
Cyproterone acetate	427-51-0	PP (1/6)	PN (0/1)	PP (1/2)	nt	nt
Daidzein	486-66-8	POS (38/38)	NEG (0/6)	POS (32/35)	POS	POS (nt/+)
Dexamethasone	50-02-2	PP (2/6)	PP (1/1)	PP (1/4)	nt	nt
Di- <i>n</i> -butyl phthalate	84-74-2	PP (5/10)	NEG (0/3)	POS (7/13)	nt	NEG (-/-)
Dibenzo[<i>a,h</i>] anthracene	53-70-3	PP (1/2)	PP (nt)	PN (0/1)	nt	nt
Dicofol*	115-32-2	POS (4/6)	NEG (0/2)	POS (2/2)	nt	nt
Diethylhexyl phthalate	117-81-7	PP (4/9)	NEG (0/3)	PP (4/8)	NEG	NEG (nt/-)
Diethylstilbestrol	56-53-1	POS (41/41)	NEG (0/2)	POS (52/52)	POS	nt
Estrone	53-16-7	POS (25/27)	PP (1/2)	POS (29/29)	POS	POS (nt/+)
Ethyl paraben	120-47-8	POS (5/5)	PN (nt)	POS (4/5)	POS	nt
Fenarimol	60168-88-9	POS (5/6)	PN (0/1)	POS (2/2)	nt	nt
Finasteride	98319-26-7	PN (nt)	PN (0/1)	PN (0/1)	nt	nt
Flavone	525-82-6	PP (2/5)	PP (1/1)	PP (3/13)	nt	nt
Fluoranthene	206-44-0	PN (nt)	PN (nt)	PN (0/1)	nt	nt
Fluoxymestron	76-43-7	PN (nt)	PN (nt)	PN (0/1)	nt	nt
Flutamide	13311-84-7	NEG (0/5)	PN (0/1)	NEG (0/2)	nt	nt
Genistein	446-72-0	POS (99/101)	NEG (0/13)	POS (64/64)	POS	POS (+/+)
Haloperidol	52-86-8	PN (0/1)	PN (nt)	PN (0/1)	nt	nt
Hydroxyflutamide	52806-53-8	NEG (0/2)	PN (nt)	PP (1/4)	nt	nt
Kaempferol	520-18-3	POS (22/22)	NEG (0/9)	POS (19/19)	POS	nt
Kepone	143-50-0	POS (13/17)	NEG (0/2)	POS (14/15)	POS	nt
Ketoconazole	65277-42-1	PN (0/1)	PN (nt)	PN (0/1)	NEG	nt
L-thyroxine	51-48-9	POS (2/3)	PN (nt)	POS (2/2)	nt	nt
Linuron	330-55-2	NEG (0/7)	PN (nt)	POS (2/3)	NEG	nt
Medroxy-progesterone acetate	71-58-9	PP (1/2)	PN (0/1)	POS (2/2)	NEG	nt
<i>meso</i> -hexestrol	84-16-2	POS (3/3)	PN (nt)	POS (11/11)	nt	nt
Methyl testosterone	58-18-4	POS (4/5)	PP (1/2)	POS (2/3)	POS	nt
Mifepristone	84371-65-3	PP (3/6)	NEG (0/3)	POS (4/6)	NEG	nt
Morin	480-16-0	PP (1/1)	PN (nt)	POS (3/3)	POS	nt
Nilutamide	63612-50-0	PN (nt)	PN (nt)	PN (nt)	nt	nt
Norethynodrel	68-23-5	POS (4/4)	NEG (2/2)	POS (7/7)	POS	na
<i>o,p'</i> -DDT	789-02-6	POS (24/25)	NEG (0/3)	POS (20/22)	nt	POS (+/nt)
Oxazepam	604-75-1	PN (nt)	PN (nt)	PN (nt)	nt	nt
<i>p</i> - <i>n</i> -nonylphenol	104-40-5	POS (9/9)	NEG (0/2)	POS (21/21)	NEG	IC (+/-)
<i>p,p'</i> -DDE	72-55-9	POS (5/7)	NEG (2/2)	PP (5/15)	nt	nt
<i>p,p'</i> -methoxychlor	72-43-5	POS (23/26)	PP (1/5)	POS (16/26)	POS	IC (+/-)
Phenobarbital	50-06-6	NEG (0/2)	PN (nt)	PN (0/1)	nt	nt
Phenolphthalin	81-90-3	PN (0/1)	PN (nt)	POS (2/2)	NEG	nt
Pimozide	2062-78-4	PN (nt)	PN (nt)	PN (nt)	nt	nt
Procymidone	32809-16-8	NEG (0/4)	PN (nt)	PP (2/5)	nt	nt
Progesterone	57-83-0	PP (3/15)	NEG (0/2)	PP (2/20)	NEG	nt
Propylthiouracil	51-52-5	PN (nt)	PN (nt)	PN (nt)	nt	nt
Raloxifene HCl*	82640-04-8	PP (7/31)	POS (13/13)	POS (16/16)	NEG	nt
Reserpine	50-55-5	PN (0/1)	PN (nt)	PN (0/1)	NEG	nt
Resveratrol*	501-36-0	POS (24/37)	NEG (0/16)	POS (9/12)	nt	nt
Sodium azide	26628-22-8	PN (0/1)	PN (nt)	PN (nt)	nt	nt
Spironolactone	52-01-7	NEG (0/3)	PN (nt)	PN (0/1)	NEG	nt
Tamoxifen	10540-29-1	POS (15/22)	POS (20/22)	POS (46/46)	POS	nt
Testosterone	58-22-0	PP (4/9)	PN (0/1)	PP (5/12)	POS	nt
Vinclozolin	50471-44-8	PP (6/13)	PN (0/1)	POS (3/5)	POS	nt

Abbreviations: CASRN = Chemical Abstracts Service Registry Number; CERI = Chemicals Evaluation and Research Institute, Japan; ER = endocrine receptor; IC = inconclusive; NEG = negative; nt = not tested; POS = positive; PP = presumed positive; PN = presumed negative; TA = transcriptional activation.

*Replacement based on list in (ICCVAM 2006)

^aValues in parentheses are the number of positive ER TA agonist studies/total number of studies (2010).

96
97
98
99
100

Do Not Cite, Quote, or Distribute

101 ^bValues in parentheses are the number of positive ER TA antagonist studies/total number of studies (2010).102 ^cValues in parentheses are the number of positive binding studies/total number of studies (2010).103 ^dChemicals Evaluation and Research Institute, Japan evaluated substances using the OECD Stably Transfected Human Estrogen Receptor- α
104 Transcriptional Activation (STTA) Assay for the Detection of Estrogenic Agonist-Activity, described in OECD Chemicals Test Guideline (TG)
105 455 (OECD 2009a; Takeyoshi 2006).106 ^eValues in parentheses are the *in vivo* uterotrophic classification using OECD study data/CERI study data (Kanno et al. 2003a, 2003b; Takeyoshi
107 2006). A consensus *in vivo* uterotrophic classification was made when OECD and CERI data were in agreement. When *in vivo* uterotrophic data
108 from OECD and CERI provided conflicting classifications, the overall classification was inconclusive.

109

110 **Table 3-3 Substances used for ER TA Agonist Assay Accuracy**

Substance	CASRN	ICCVAM Consensus Classification	Mean EC ₅₀ ^a (M)
17 α -Estradiol	57-91-0	POS	1.92 \times 10 ⁻⁷
17 α -Ethinyl estradiol	57-63-6	POS	2.44 \times 10 ⁻⁹
17 β -Estradiol	50-28-2	POS	1.33 \times 10 ⁻⁸
19-Nortestosterone	434-22-0	POS	1.30 \times 10 ⁻⁷
4-cumylphenol	599-64-4	POS	3.22 \times 10 ⁻⁷
4- <i>tert</i> -octylphenol	140-66-9	POS	4.54 \times 10 ⁻⁶
5 α -dihydrotestosterone	521-18-6	POS	2.50 \times 10 ⁻⁷
Apigenin	520-36-5	POS	7.64 \times 10 ⁻⁷
Atrazine	1912-24-9	NEG	n.a.
Bicalutamide	90357-06-5	NEG	n.a.
Bisphenol A	80-05-7	POS	3.69 \times 10 ⁻⁶
Bisphenol B	77-40-7	POS	4.18 \times 10 ⁻⁵
Butylbenzyl phthalate	85-68-7	POS	5.10 \times 10 ⁻⁶
Chrysin	480-40-0	POS	n.a.
Clomiphene citrate	50-41-9	POS	5.00 \times 10 ⁻⁹
Corticosterone	50-22-6	NEG	n.a.
Coumestrol	479-13-0	POS	2.00 \times 10 ⁻⁷
Daidzein	486-66-8	POS	3.05 \times 10 ⁻⁶
Dicofol	115-32-2	POS	7.05 \times 10 ⁻⁶
Diethylstilbestrol	56-53-1	POS	1.29 \times 10 ⁻⁷
Estrone	53-16-7	POS	8.33 \times 10 ⁻⁸
Ethyl paraben	120-47-8	POS	5.00 \times 10 ⁻⁵
Fenarimol	60168-88-9	POS	7.00 \times 10 ⁻⁶
Flutamide	13311-84-7	NEG	n.a.
Genistein	446-72-0	POS	1.66 \times 10 ⁻⁵
Hydroxy Flutamide	52806-53-8	NEG	n.a.
Kaempferol	520-18-3	POS	1.60 \times 10 ⁻⁷
Kepone	143-50-0	POS	nc
L-Thyroxine	51-48-9	POS	5.00 \times 10 ⁻⁹

Do Not Cite, Quote, or Distribute

Substance	CASRN	ICCVAM Consensus Classification	Mean EC ₅₀ ^a (M)
Linuron	330-55-2	NEG	n.a.
<i>meso</i> -Hexestrol	84-16-2	POS	1.13×10^{-10}
Methyl testosterone	58-18-4	POS	1.38×10^{-6}
Norethynodrel	68-23-5	POS	6.59×10^{-8}
<i>o,p'</i> -DDT	789-02-6	POS	1.67×10^{-4}
<i>p</i> -n-nonylphenol	104-40-5	POS	1.59×10^{-6}
<i>p,p'</i> -methoxychlor	72-43-5	POS	1.56×10^{-4}
<i>p,p'</i> -DDE	72-55-9	POS	3.00×10^{-6}
Phenobarbital	50-06-6	NEG	n.a.
Procymidone	32809-16-8	NEG	n.a.
Resveratrol	501-36-0	POS	7.86×10^{-6}
Spirolactone	52-01-7	NEG	n.a.
Tamoxifen	10540-29-1	POS	1.35×10^{-6}

111 Abbreviations: EC₅₀ = the half maximal effective concentration; n.a. = not applicable; nc = not calculated; NEG = negative; POS
 112 = positive.

113 ^a Mean EC₅₀ calculated from values reported in the literature.

114

115 **Table 3-4 Substances Used for ER TA Antagonist Assay Accuracy**

Substance	CASRN	ICCVAM Consensus Classification	Mean IC ₅₀ ^a (M)
17 α -ethinyl estradiol	57-63-6	NEG	n.a.
4-Hydroxytamoxifen	68047-06-3	POS	1.93×10^{-8}
5 α -Dihydrotestosterone	521-18-6	NEG	n.a.
Apigenin	520-36-5	NEG	n.a.
Bisphenol A	80-05-7	NEG	n.a.
Butylbenzyl phthalate	85-68-7	NEG	n.a.
Chrysin	480-40-0	NEG	n.a.
Coumestrol	479-13-0	NEG	n.a.
Daidzein	486-66-8	NEG	n.a.
Di- <i>n</i> -butyl phthalate	84-74-2	NEG	n.a.
Dicofol	115-32-2	NEG	n.a.
Diethylhexyl phthalate	117-81-7	NEG	n.a.
Diethylstilbestrol	56-53-1	NEG	n.a.
Genistein	446-72-0	NEG	n.a.
Kaempferol	520-18-3	NEG	n.a.
Kepone	143-50-0	NEG	n.a.

Do Not Cite, Quote, or Distribute

Mifepristone	84371-65-3	NEG	n.a.
Norethynodrel	68-23-5	NEG	n.a.
<i>o,p'</i> -DDT	789-02-6	NEG	n.a.
<i>p</i> -n-nonylphenol	104-40-5	NEG	n.a.
<i>p,p'</i> -DDE	72-55-9	NEG	n.a.
Progesterone	57-83-0	NEG	n.a.
Raloxifene HCl	82640-04-8	POS	6.23×10^{-8}
Resveratrol	501-36-0	NEG	n.a.
Tamoxifen	10540-29-1	POS	1.26×10^{-6}

116 Abbreviations: IC₅₀ = half maximal inhibitory concentration; n.a. = not applicable; NEG = negative; POS = positive.

117 ^a Mean IC₅₀ calculated from values reported in the literature.

118

119 3.3 Substances Used to Evaluate Concordance with Other Accepted Methods

120 The primary evaluation of accuracy described in **Section 6.0** is based on a comparison of the test
 121 substance classification by the BG1Luc ER TA to the ICCVAM reference classification of that substance,
 122 as outlined in **Section 3.2**. However, concordance with other methods currently accepted by regulators to
 123 evaluate estrogenic activity was also considered in this evaluation. Among these, the most commonly
 124 used are: the *in vitro* Stably Transfected Transactivation Assay (STTA) by the Japanese Chemicals
 125 Evaluation and Research Institute (CERI) using the hER α - HeLa-9903 cell line (CERI-STTA) test
 126 method for ER agonists, *in vitro* ER binding assays, and the *in vivo* rodent uterotrophic bioassay. The
 127 substances used in the concordance analyses with each of these methods, and the rationale for their
 128 selection, are detailed below in **Sections 3.3.1 to 3.3.3**.

129 3.3.1 Substances Used to Evaluate ER TA Assay Concordance with the CERI-STTA 130 Test Method

131 The *in vitro* assessment of ER TA activity is included in Tier 1 of the EPA's EDSP screening battery, and
 132 has been incorporated into the OECD Conceptual Framework for the Testing and Assessment of
 133 Endocrine Disrupting Chemicals" as "Level 2" assays to provide mechanistic information for the purpose
 134 of testing prioritization. At present, there is only one *in vitro* ER TA test method that is considered to be
 135 regulatory accepted for identifying substances with potential ER agonist activity (CERI-STTA). This test
 136 method has recently been adopted in the U.S. as EPA Health Effects Test Guideline 890.1300 (EPA
 137 2009) (EPA 2009), and internationally as OECD Test Guideline 455 (OECD 2009b). The hER α -HeLa-
 138 9903 cell line is derived from a human cervical tumor, with two stably inserted constructs: (i) the hER α
 139 expression construct (encoding the full-length human receptor), and (ii) a firefly luciferase reporter

Do Not Cite, Quote, or Distribute

140 construct bearing five tandem repeats of a vitellogenin Estrogen-Responsive Element (ERE) driven by a
141 mouse metallothionein (MT) promoter TATA element (OECD 2009b).

142 There were 41 substances common to both the BG1Luc ER TA and CERI-STTA validation studies.
143 CERI-STTA data (ER TA agonist classifications) for these 41 reference substances are included in **Table**
144 **3-2**. Based on these available data, ICCVAM conducted a direct comparison of concordance between
145 agonist classifications based on data from the BG1Luc ER TA and CERI-STTA validation studies
146 (**Section 5**).

147 **3.3.2 Substances Used to Evaluate EA TA Assay Concordance with *In Vitro* ER** 148 **Binding Test Methods**

149 The *in vitro* assessment of ER binding is included in Tier 1 of the EPA’s EDSP screening battery, and has
150 been incorporated into the OECD Conceptual Framework for the Testing and Assessment of Endocrine
151 Disrupting Chemicals” as “Level 2” assays to provide mechanistic information for the purpose of testing
152 prioritization. *In vitro* ER binding assays identify substances that can bind to the ER, whereas *in vitro* ER
153 TA assays measure the ability of a test substance to activate or inhibit the transactivation of a reporter
154 gene via ER mediated pathways. Accordingly, the ability of a test substance to bind to the ER *in vitro*
155 suggests (but does not demonstrate) the ability of the substance to activate or inhibit *in vitro* ER mediated
156 transactivation, and vice-versa. In order to determine the extent of agreement between the BG1Luc ER
157 TA and ER binding data, ICCVAM conducted a concordance evaluation using data from the BG1Luc ER
158 TA validation study and published ER binding data (**Section 5**).

159 Classification of the reference substances with respect to *in vitro* ER binding was based on a
160 preponderance of evidence found in a review of the scientific literature, as described for EA TA assays in
161 **Section 3.2**. Relevant information from 67 publications describing *in vitro* ER binding data was extracted
162 and is provided in **Annex N**.

163 ICCVAM used the following criteria to classify each reference substance:

- 164 • A substance was classified as Positive (POS) if it was reported as positive in > 50% of
165 referenced ER binding studies.
- 166 • A substance was classified as Negative (NEG) if it was reported as negative in all referenced
167 ER binding studies (minimum of two studies were required for Negative classification).
- 168 • A substance was classified as Presumed Positive (PP) if it was positive in 50% or less of
169 referenced ER binding studies, or if it was reported positive in the single study conducted.
- 170 • A substance was classified as Presumed Negative (PN) if it was reported negative in a single
171 ER binding study.

Do Not Cite, Quote, or Distribute

- 172 • Substances without data were classified as PP or PN based on other available information,
173 including their known mechanism of action or their responses in other ER assays.

174 A summary of the ER binding literature data for all ICCVAM reference substances is provided in **Table**
175 **3-2**, along with the resulting ER binding classifications.

176 **3.3.3 Substances Used to Evaluate EA TA Assay Concordance with the *In Vivo*** 177 **Uterotrophic Bioassay**

178 As stated in OECD TG 440, the uterotrophic bioassay is a short-term screening test that evaluates the
179 ability of a substance to elicit estrogenic activity (Kanno et al. 2003a, 2003b; OECD 2007; Owens and
180 Ashby 2002; Owens and Koeter 2003). In this *in vivo* test method, the uterus responds to estrogens
181 initially with an increase in weight resulting from water inhibition, followed by further weight gain due to
182 increased tissue growth. The uterotrophic bioassay is included in Level 3 of the “OECD Conceptual
183 Framework for the Testing and Assessment of Endocrine Disrupting Chemicals” as an *in vivo* assay
184 providing data about estrogenicity. The rat uterotrophic bioassay is also included as one of the *in vivo*
185 methods in the EPA’s EDSP Tier 1 screening battery. In order to determine the extent of agreement
186 between the BG1Luc ER TA and the rat uterotrophic bioassay, ICCVAM conducted a concordance
187 evaluation using data from the BG1Luc ER TA validation study and published uterotrophic bioassay data
188 (**Section 5**).

189 Classification of the reference substances with respect to *in vivo* rodent uterotrophic activity was based on
190 data from studies sponsored by the OECD (OECD 2007) and studies that were conducted in conjunction
191 CERI (ER TA assay validation studies (Kanno et al. 2003b)). Combined, these studies tested 15
192 substances from the list of 78 ICCVAM reference substances. The *in vivo* uterotrophic data used to
193 compare BG1Luc ER TA validation study agonist results were selected using the following criteria:

- 194 • Substances that tested positive in both the OECD and CERI studies (three substances)
195 • Substances that tested negative in both the OECD and CERI studies (two substance)
196 • Substances that tested positive or negative in at least one OECD or CERI study but that were
197 not tested in both studies (seven positive and one negative)
198 • Substances that tested positive in one study but negative in the other were defined as
199 “inconclusive” and were not used in the comparison.

200 Classification of the 15 reference substances with respect to uterotrophic activity is provided in
201 **Table 3-2**.

Do Not Cite, Quote, or Distribute

202 **3.4 Substances Tested in Each Phase of Validation.**

203 As described in **Section 2.0**, the test method validation was conducted in four consecutive phases in order
 204 to identify and resolve sources of variation early in the validation process. Substances used in each phase
 205 of the agonist and antagonist testing are listed in **Tables 3-5** and **Table 3-6**, respectively.

206 **Table 3-5 Testing Phases for ER TA Agonists**

Study Phase	Substance	CASRN	MESH Chemical Class ^a	Product Class ^b	ICCVAM Consensus Classification
1	17 β -Estradiol	50-28-2	Steroid	Pharmaceutical, Veterinary Agent	POS
1	<i>p,p'</i> -Methoxychlor	72-43-5	Hydrocarbon (Halogenated)	Pesticide, Veterinary Agent	POS
2a	Bisphenol A	80-05-7	Phenol	Chemical Intermediate, Flame Retardant, Fungicide	POS
2a	Bisphenol B	77-40-7	Phenol	Chemical Intermediate, Flame Retardant, Fungicide	POS
2a	Corticosterone	50-22-6	Steroid	Pharmaceutical	PP
2a	Diethylstilbestrol	56-53-1	Hydrocarbon (Cyclic)	Pharmaceutical, Veterinary Agent	POS
2b	17 α -Ethinyl estradiol	57-63-6	Steroid	Pharmaceutical, Veterinary Agent	POS
2b	Atrazine	1912-24-9	Heterocyclic Compound	Herbicide	NEG
2b	Butylbenzyl phthalate	85-68-7	Carboxylic Acid, Phthalic Acid	Pharmaceutical, Veterinary Agent	POS
2b	Flavone	525-82-6	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical	PP
2b	Genistein	446-72-0	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical	POS

Do Not Cite, Quote, or Distribute

Study Phase	Substance	CASRN	MESH Chemical Class ^a	Product Class ^b	ICCVAM Consensus Classification
2b	<i>o,p'</i> -DDT	789-02-6	Hydrocarbon (Halogenated)	Pesticide	POS
2b	<i>p-n</i> -Nonylphenol	104-40-5	Phenol	Chemical Intermediate	POS
2b	Vinclozolin	50471-44-8	Heterocyclic Compound	Fungicide	PP
3	12- <i>O</i> -Tetradecanoylphorbol-13-acetate	16561-29-8	Hydrocarbon (Cyclic)	Laboratory Chemical	PN
3	17 β -Estradiol	50-28-2	Steroid	Pharmaceutical, Veterinary Agent	POS
3	17 α -Estradiol	57-91-0	Steroid	Pharmaceutical, Veterinary Agent	POS
3	2-sec-Butylphenol	89-72-5	Phenol	Chemical Intermediate, Pesticide Intermediate, Plasticizer	NEG
3	2,4,5-Trichlorophenoxyacetic acid	93-76-5	Carboxylic Acid	Herbicide	PP
3	4-Androstenedione	63-05-8	Steroid	Pharmaceutical	PP
3	4-Cumylphenol	599-64-4	Phenol	Chemical Intermediate	POS
3	4-Hydroxytamoxifen	68047-06-3	Hydrocarbon (Cyclic)	Pharmaceutical	PP
3	4-tert-Octylphenol	140-66-9	Phenol	Chemical Intermediate, Pharmaceutical Intermediate	POS
3	5 α -Dihydro-testosterone	521-18-6	Steroid	Pharmaceutical	POS
3	Actinomycin D	50-76-0	Heterocyclic Compound, Polycyclic Compound	Laboratory Chemical, Pharmaceutical, Veterinary Agent	PN

Do Not Cite, Quote, or Distribute

Study Phase	Substance	CASRN	MESH Chemical Class ^a	Product Class ^b	ICCVAM Consensus Classification
3	Apigenin	520-36-5	Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate	POS
3	Clomiphene citrate	50-41-9	Amine, Carboxylic Acid, Heterocyclic Compound	Pharmaceutical	POS
3	Coumestrol	479-13-0	Heterocyclic Compound	Natural Product	POS
3	Daidzein	486-66-8	Flavonoid, Heterocyclic Compound	Natural Product	POS
3	Dexamethasone	50-02-2	Steroid	Pharmaceutical, Veterinary Agent	PP
3	Di- <i>n</i> -butyl phthalate	84-74-2	Ester, Phthalic Acid	Cosmetic Ingredient, Industrial Chemical, Plasticizer	POS
3	Dibenzo[<i>a,h</i>] Anthracene	53-70-3	Polycyclic Compound	Laboratory Chemical, Natural Product	PP
3	Dicofol	115-32-2	Hydrocarbon (Cyclic), Hydrocarbon (Halogenated)	Pesticide	POS
3	Diethylhexyl phthalate	117-81-7	Phthalic Acid	Pesticide Intermediate, Plasticizer	PP
3	Estrone	53-16-7	Steroid	Pharmaceutical, Veterinary Agent	POS
3	Ethyl paraben	120-47-8	Carboxylic Acid, Phenol	Pharmaceutical, Preservative	POS
3	Fluoranthene	206-44-0	Polycyclic Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical Intermediate	PN

Do Not Cite, Quote, or Distribute

Study Phase	Substance	CASRN	MESH Chemical Class ^a	Product Class ^b	ICCVAM Consensus Classification
3	Hydroxy flutamide	52806-53-8	Amide	Pharmaceutical	NEG
3	Kaempferol	520-18-3	Flavonoid, Heterocyclic Compound	Natural Product	POS
3	Kepone	143-50-0	Hydrocarbon (Halogenated)	Pesticide	POS
3	meso-Hexestrol	84-16-2	Steroid	Pharmaceutical, Veterinary Agent	PP
3	Methyl testosterone	58-18-4	Steroid	Pharmaceutical, Veterinary Agent	POS
3	Morin	480-16-0	Flavonoid, Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate	POS
3	Norethynodrel	68-23-5	Steroid	Pharmaceutical	POS
3	<i>p,p'</i> -Methoxychlor	72-43-5	Hydrocarbon (Halogenated)	Pesticide, Veterinary Agent	POS
3	<i>p,p'</i> -DDE	72-55-9	Hydrocarbon (Halogenated)	Pesticide Intermediate	POS
3	Phenobarbital	50-06-6	Heterocyclic Compound, Pyrimidine	Pharmaceutical, Veterinary Agent	NEG
3	Phenolphthalin	81-90-3	Carboxylic Acid, Phenol	Dye, Laboratory Chemical	NEG
3	Progesterone	57-83-0	Steroid	Pharmaceutical, Veterinary Agent	PP
3	Propylthiouracil	51-52-5	Heterocyclic Compound, Pyrimidine	Pharmaceutical, Veterinary Agent	PN
3	Raloxifene HCl	82640-04-8	Hydrocarbon (Cyclic)	Pharmaceutical	PP
3	Resveratrol	501-36-0	Hydrocarbon (Cyclic)	Natural Product	POS

Do Not Cite, Quote, or Distribute

Study Phase	Substance	CASRN	MESH Chemical Class ^a	Product Class ^b	ICCVAM Consensus Classification
3	Sodium azide	26628-22-8	Azide, Salt (Inorganic)	Chemical Intermediate, Fungicide, Herbicide	PN
3	Tamoxifen	10540-29-1	Hydrocarbon (Cyclic)	Pharmaceutical	POS
3	Testosterone	58-22-0	Steroid	Pharmaceutical, Veterinary Agent	PP
4	17 β -Trenbolone	10161-33-8	Steroid	Pharmaceutical	POS
4	19-Nortestosterone	434-22-0	Steroid	Pharmaceutical, Veterinary Agent	POS
4	4-Hydroxy-androstenedione	566-48-3	Steroid	Pharmaceutical	PP
4	Ammonium perchlorate	7790-98-9	Amine, Onium Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical	PN
4	Apomorphine	58-00-4	Heterocyclic Compound	Pharmaceutical, Veterinary Agent	PN
4	Bicalutamide	90357-06-5	Amide	Pharmaceutical	NEG
4	Chrysin	480-40-0	Flavonoid, Heterocyclic Compound	Natural Product	POS
4	Cycloheximide	66-81-9	Heterocyclic Compound	Fungicide, Pharmaceutical, Veterinary Agent	PN
4	Cyproterone acetate	427-51-0	Steroid	Pharmaceutical	PP
4	Fenarimol	60168-88-9	Heterocyclic Compound, Pyrimidine	Fungicide	POS
4	Finasteride	98319-26-7	Steroid	Pharmaceutical	PN
4	Fluoxymestron	76-43-7	Steroid	Pharmaceutical	PN

Do Not Cite, Quote, or Distribute

Study Phase	Substance	CASRN	MESH Chemical Class ^a	Product Class ^b	ICCVAM Consensus Classification
4	Flutamide	13311-84-7	Amide	Pharmaceutical, Veterinary Agent	NEG
4	Haloperidol	52-86-8	Ketone	Pharmaceutical, Veterinary Agent	NEG
4	Ketoconazole	65277-42-1	Heterocyclic Compound	Pharmaceutical	NEG
4	L-Thyroxine	51-48-9	Amino Acid	Pharmaceutical, Veterinary Agent	POS
4	Linuron	330-55-2	Urea	Herbicide	NEG
4	Medroxyprogesterone acetate	71-58-9	Steroid	Pharmaceutical	POS
4	Mifepristone	84371-65-3	Steroid	Pharmaceutical	POS
4	Nilutamide	63612-50-0	Heterocyclic Compound, Imidazole	Pharmaceutical	PN
4	Oxazepam	604-75-1	Heterocyclic Compound	Pharmaceutical, Veterinary Agent	PN
4	Pimozide	2062-78-4	Heterocyclic Compound	Pharmaceutical	PN
4	Procymidone	32809-16-8	Polycyclic Compound	Fungicide	NEG
4	Reserpine	50-55-5	Heterocyclic Compound, Indole	Pharmaceutical, Veterinary Agent	PN
4	Spirolactone	52-01-7	Lactone, Steroid	Pharmaceutical	NEG

207 Abbreviations: NEG = negative; PN = presumed negative; POS = positive; PP = presumed positive.

208 ^aSubstances were assigned into one or more chemical classes using the U.S. National Library of Medicine's Medical Subject
209 Headings (MeSH), an internationally recognized standardized classification scheme (available at: <http://www.nlm.nih.gov/mesh>).

210 ^bSubstances were assigned into one or more product classes using the U.S. National Library of Medicine's Hazardous Substances
211 Database (available at: <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>).

212

213

Do Not Cite, Quote, or Distribute

213

214 **Table 3-6 Testing Phases for ER TA Antagonists**

Study Phase	Substance	CASRN	MESH Chemical Class ^a	Product Class ^b	ICCVAM Consensus Classification
1	17 β -Estradiol	50-28-2	Steroid	Pharmaceutical, Veterinary Agent	PP
1	Flavone	525-82-6	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical	PP
1	Raloxifene HCl	82640-04-8	Hydrocarbon (Cyclic)	Pharmaceutical	POS
2a	Dibenzo[<i>a,h</i>] Anthracene	53-70-3	Polycyclic Compound	Laboratory Chemical, Natural Product	PP
2a	<i>p-n</i> -Nonylphenol	104-40-5	Phenol	Chemical Intermediate	PP
2a	Progesterone	57-83-0	Steroid	Pharmaceutical, Veterinary Agent	PP
2a	Tamoxifen	10540-29-1	Hydrocarbon (Cyclic)	Pharmaceutical	POS
2b	Apigenin	520-36-5	Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate	PP
2b	Atrazine	1912-24-9	Heterocyclic Compound	Herbicide	NEG
2b	Butylbenzyl phthalate	85-68-7	Carboxylic Acid, Phthalic Acid	Pharmaceutical, Veterinary Agent	NEG
2b	Corticosterone	50-22-6	Steroid	Pharmaceutical	PP
2b	Flavone	525-82-6	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical	PP
2b	Genistein	446-72-0	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical	PP
2b	<i>o,p'</i> -DDT	789-02-6	Hydrocarbon (Halogenated)	Pesticide	PP

Do Not Cite, Quote, or Distribute

Study Phase	Substance	CASRN	MESH Chemical Class ^a	Product Class ^b	ICCVAM Consensus Classification
2b	Resveratrol	501-36-0	Hydrocarbon (Cyclic)	Natural Product	POS
3	12- <i>O</i> -Tetradecanoylphorbol-13-acetate	16561-29-8	Hydrocarbon (Cyclic)	Laboratory Chemical	PN
3	17 β -Estradiol	50-28-2	Steroid	Pharmaceutical, Veterinary Agent	PP
3	17 α -Estradiol	57-91-0	Steroid	Pharmaceutical, Veterinary Agent	PP
3	17 α -Ethinyl estradiol	57-63-6	Steroid	Pharmaceutical, Veterinary Agent	PP
3	2- <i>sec</i> -Butylphenol	89-72-5	Phenol	Chemical Intermediate, Pesticide Intermediate, Plasticizer	PN
3	2,4,5-Trichlorophenoxyacetic acid	93-76-5	Carboxylic Acid	Herbicide	PP
3	4-Androstenedione	63-05-8	Steroid	Pharmaceutical	NEG
3	4-Cumylphenol	599-64-4	Phenol	Chemical Intermediate	PN
3	4-Hydroxytamoxifen	68047-06-3	Hydrocarbon (Cyclic)	Pharmaceutical	POS
3	4- <i>tert</i> -Octylphenol	140-66-9	Phenol	Chemical Intermediate, Pharmaceutical Intermediate	PN
3	5 α -Dihydrotestosterone	521-18-6	Steroid	Pharmaceutical	NEG
3	Actinomycin D	50-76-0	Heterocyclic Compound, Polycyclic Compound	Laboratory Chemical, Pharmaceutical, Veterinary Agent	PN

Do Not Cite, Quote, or Distribute

Study Phase	Substance	CASRN	MESH Chemical Class ^a	Product Class ^b	ICCVAM Consensus Classification
3	Bisphenol A	80-05-7	Phenol	Chemical Intermediate, Flame Retardant, Fungicide	PP
3	Bisphenol B	77-40-7	Phenol	Chemical Intermediate, Flame Retardant, Fungicide	NEG
3	Clomiphene citrate	50-41-9	Amine, Carboxylic Acid, Heterocyclic Compound	Pharmaceutical	PP
3	Coumestrol	479-13-0	Heterocyclic Compound	Natural Product	PP
3	Daidzein	486-66-8	Flavonoid, Heterocyclic Compound	Natural Product	NEG
3	Dexamethasone	50-02-2	Steroid	Pharmaceutical, Veterinary Agent	PP
3	Di- <i>n</i> -butyl phthalate	84-74-2	Ester, Phthalic Acid	Cosmetic Ingredient, Industrial Chemical, Plasticizer	PP
3	Dicofol	115-32-2	Hydrocarbon (Cyclic), Hydrocarbon (Halogenated)	Pesticide	PP
3	Diethylhexyl phthalate	117-81-7	Phthalic Acid	Pesticide Intermediate, Plasticizer	PP
3	Diethylstilbestrol	56-53-1	Hydrocarbon (Cyclic)	Pharmaceutical, Veterinary Agent	PP
3	Estrone	53-16-7	Steroid	Pharmaceutical, Veterinary Agent	PP
3	Ethyl paraben	120-47-8	Carboxylic Acid, Phenol	Pharmaceutical, Preservative	PN

Do Not Cite, Quote, or Distribute

Study Phase	Substance	CASRN	MESH Chemical Class ^a	Product Class ^b	ICCVAM Consensus Classification
3	Fluoranthene	206-44-0	Polycyclic Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical Intermediate	PN
3	Hydroxy Flutamide	52806-53-8	Amide	Pharmaceutical	PN
3	Kaempferol	520-18-3	Flavonoid, Heterocyclic Compound	Natural Product	PP
3	Kepone	143-50-0	Hydrocarbon (Halogenated)	Pesticide	NEG
3	<i>meso</i> -Hexestrol	84-16-2	Steroid	Pharmaceutical, Veterinary Agent	PN
3	Methyl testosterone	58-18-4	Steroid	Pharmaceutical, Veterinary Agent	PP
3	Morin	480-16-0	Flavonoid, Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate	PN
3	Norethynodrel	68-23-5	Steroid	Pharmaceutical	NEG
3	<i>p,p'</i> -Methoxychlor	72-43-5	Hydrocarbon (Halogenated)	Pesticide, Veterinary Agent	NEG
3	<i>p,p'</i> -DDE	72-55-9	Hydrocarbon (Halogenated)	Pesticide Intermediate	NEG
3	Phenobarbital	50-06-6	Heterocyclic Compound, Pyrimidine	Pharmaceutical, Veterinary Agent	PN
3	Phenolphthalin	81-90-3	Carboxylic Acid, Phenol	Dye, Laboratory Chemical	PN
3	Propylthiouracil	51-52-5	Heterocyclic Compound, Pyrimidine	Pharmaceutical, Veterinary Agent	PN
3	Raloxifene HCl	82640-04-8	Hydrocarbon (Cyclic)	Pharmaceutical	POS

Do Not Cite, Quote, or Distribute

Study Phase	Substance	CASRN	MESH Chemical Class ^a	Product Class ^b	ICCVAM Consensus Classification
3	Sodium azide	26628-22-8	Azide, Salt (Inorganic)	Chemical Intermediate, Fungicide, Herbicide	PN
3	Testosterone	58-22-0	Steroid	Pharmaceutical, Veterinary Agent	PP
3	Vinclozolin	50471-44-8	Heterocyclic Compound	Fungicide	PP
4	17 β -Trenbolone	10161-33-8	Steroid	Pharmaceutical	PN
4	19-Nortestosterone	434-22-0	Steroid	Pharmaceutical, Veterinary Agent	PP
4	4-Hydroxy-androstenedione	566-48-3	Steroid	Pharmaceutical	PN
4	Ammonium perchlorate	7790-98-9	Amine, Onium Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical	PN
4	Apomorphine	58-00-4	Heterocyclic Compound	Pharmaceutical, Veterinary Agent	PN
4	Bicalutamide	90357-06-5	Amide	Pharmaceutical	PN
4	Chrysin	480-40-0	Flavonoid, Heterocyclic Compound	Natural Product	PP
4	Cycloheximide	66-81-9	Heterocyclic Compound	Fungicide, Pharmaceutical, Veterinary Agent	PN
4	Cyproterone acetate	427-51-0	Steroid	Pharmaceutical	NEG
4	Fenarimol	60168-88-9	Heterocyclic Compound, Pyrimidine	Fungicide	NEG
4	Finasteride	98319-26-7	Steroid	Pharmaceutical	NEG
4	Fluoxymestron	76-43-7	Steroid	Pharmaceutical	PN

Do Not Cite, Quote, or Distribute

Study Phase	Substance	CASRN	MESH Chemical Class ^a	Product Class ^b	ICCVAM Consensus Classification
4	Flutamide	13311-84-7	Amide	Pharmaceutical, Veterinary Agent	NEG
4	Haloperidol	52-86-8	Ketone	Pharmaceutical, Veterinary Agent	PN
4	Ketoconazole	65277-42-1	Heterocyclic Compound	Pharmaceutical	PN
4	L-Thyroxine	51-48-9	Amino Acid	Pharmaceutical, Veterinary Agent	PN
4	Linuron	330-55-2	Urea	Herbicide	PN
4	Medroxyprogesterone acetate	71-58-9	Steroid	Pharmaceutical	NEG
4	Mifepristone	84371-65-3	Steroid	Pharmaceutical	PP
4	Nilutamide	63612-50-0	Heterocyclic Compound, Imidazole	Pharmaceutical	PN
4	Oxazepam	604-75-1	Heterocyclic Compound	Pharmaceutical, Veterinary Agent	PN
4	Pimozide	2062-78-4	Heterocyclic Compound	Pharmaceutical	PN
4	Procymidone	32809-16-8	Polycyclic Compound	Fungicide	PN
4	Reserpine	50-55-5	Heterocyclic Compound, Indole	Pharmaceutical, Veterinary Agent	PN
4	Spironolactone	52-01-7	Lactone, Steroid	Pharmaceutical	PN

215 Abbreviations: NEG = negative; PN = presumed negative; POS = positive; PP = presumed positive.

216 ^aSubstances were assigned into one or more chemical classes using the U.S. National Library of Medicine's Medical Subject
217 Headings (MeSH), an internationally recognized standardized classification scheme (available at: <http://www.nlm.nih.gov/mesh>).

218 ^bSubstances were assigned into one or more product classes using the U.S. National Library of Medicine's Hazardous Substances
219 Database (available at: <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>).

220

Do Not Cite, Quote, or Distribute

221 **3.5 Substances Used for Intralaboratory Reproducibility**

222 Intralaboratory reproducibility was assessed using data generated by testing the 12 coded reference
223 substances in Phase 2, which were each tested three times on three separate days. The substances tested in
224 Phase 2 that were used to assess intralaboratory reproducibility of the agonist and antagonist test methods
225 are listed in **Tables 3-5** and **Table 3-6**, respectively.

226 **3.6 Substances Used to Assess Interlaboratory Reproducibility**

227 Since this validation study was conducted using a phased approach (**Section 3.1**), not all substances were
228 tested in all labs. Consequently, only those coded substances tested in all three laboratories (Phase 2 and
229 Phase 3) could be used to assess interlaboratory reproducibility. The 53 substances tested in Phase 2 and
230 Phase 3 that were used to assess interlaboratory reproducibility of the agonist and antagonist test methods
231 are listed in **Table 3-5** and **Table 3-6**, respectively.

232 **3.7 Chemical Classes Represented by the List of Substances**

233 The chemical classes assigned to each reference substance are based on a chemical classification system
234 consistent with the U.S. National Library of Medicine’s Medical Subject Headings (MeSH[®]; available at:
235 <http://www.nlm.nih.gov/mesh>), an internationally recognized standardized classification scheme. The
236 distribution of substances by chemical class is provided in **Table 3-7**.

237 **Table 3-7 List of 78 Substances: Distribution by Chemical Class**

MeSH Chemical Classes ^a	All Substances	Substances used for Agonist Accuracy	Substances used for Antagonist Accuracy
Amides	3	3	1
Amines	2	1	0
Amino Acids	1	1	0
Azides	1	0	0
Carboxylic Acids	5	4	1
Esters	2	0	0
Flavonoids	8	7	1
Heterocyclic Compounds	22	12	3
Hydrocarbons (Cyclic)	7	4	2
Hydrocarbons	5	5	3

Do Not Cite, Quote, or Distribute

MeSH Chemical Classes ^a	All Substances	Substances used for Agonist Accuracy	Substances used for Antagonist Accuracy
(Halogenated)			
Imidazoles	1	0	0
Indoles	1	0	0
Ketones	1	1	0
Lactones	1	1	0
Onium Compounds	1	0	0
Phenols	8	8	1
Phthalic Acids	3	1	1
Polycyclic Compounds	4	1	0
Pyrimidines	3	2	1
Salts (Inorganic)	1	1	0
Steroids	22	12	5
Ureas	1	1	0

238 ^aSubstances were assigned into one or more chemical classes using the U.S. National Library of Medicine's Medical Subject
 239 Headings (MeSH), an internationally recognized standardized classification scheme (available at: <http://www.nlm.nih.gov/mesh>).

240

241 3.8 Product Classes Represented by the List of Substances

242 The product classes assigned to each reference substance are based on information obtained from the U.S.

243 National Library of Medicine's Hazardous Substances Database (available at:

244 <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>). For *in vitro* ER test methods, the distribution of

245 substances by product class is provided in **Table 3-8**.

246

Do Not Cite, Quote, or Distribute

246

247 **Table 3-8 List of 78 Substances: Distribution by Product Class**

Product Classes ^a	All Substances	Substances use for Agonist Accuracy	Substances use for Antagonist Accuracy
Chemical Intermediate	7	7	1
Cosmetic Ingredient	1	0	0
Dye	3	3	0
Flame Retardant	2	2	1
Fungicide	7	5	2
Herbicide	4	3	1
Industrial Chemical	4	0	0
Laboratory Chemical	6	1	0
Natural Product	10	8	1
Pesticide	4	4	2
Pesticide Intermediate	3	2	1
Pharmaceutical	46	25	10
Pharmaceutical Intermediate	4	3	0
Plasticizer	4	1	0
Preservative	1	1	0
Veterinary Agent	22	13	3

248 ^aSubstances were assigned into one or more product classes using the U.S. National Library of Medicine's Hazardous Substances
 249 Database (available at: <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>).

250

251 3.9 Test Substance Procurement, Coding, and Distribution

252 On behalf of NICEATM, the National Toxicology Program Substance Inventory (NTPSI) procured and
 253 distributed all reference standards and controls to the participating laboratories with the exception of some
 254 that were classified as “controlled substances” (i.e., 4-androstenedione, 5 α -dihydrotestosterone, methyl
 255 testosterone, testosterone, and phenobarbital). To avoid the extensive amount of documentation required
 256 (and associated time delays) for the importation of controlled substances, ECVAM and JaCVAM made
 257 efforts to procure these specific substances from their regional suppliers. ECVAM procured methyl
 258 testosterone and phenobarbital from EU based suppliers but not 4-androstenedione, 5 α -

Do Not Cite, Quote, or Distribute

259 dihydrotestosterone, and testosterone. Therefore, ECVAM obtained the required EU regulatory
260 permissions for the importation of 4-androstenedione, 5 α -dihydrotestosterone, and testosterone, which
261 were subsequently procured by the NTPSI and exported to the ECVAM laboratory accordingly. JaCVAM
262 was able to procure 4-androstenedione, 5 α -dihydrotestosterone, methyl testosterone, and testosterone
263 from Japanese suppliers. However, phenobarbital, classified as a schedule IV controlled substance
264 according to the U.S. Drug Enforcement Administration, was not procured because the JaCVAM-
265 sponsored Hiyoshi laboratory did not have an appropriate license for handling schedule IV substances.

266 Reference substances were coded with a laboratory-specific unique identifier (see **Annex I** for laboratory
267 specific reference substance codes), and aliquots were sent in coded vials to participating laboratories
268 (**note:** the NTPSI also provided empty coded vials to ECVAM and JaCVAM for the controlled
269 substances that were procured from regional distributors as detailed above). Reference standards and
270 controls were provided with material safety data sheets (MSDS) and coded reference substances were
271 provided with a sealed envelope containing the identity of each test substance as well as its MSDS to be
272 opened in the event an accident occurred (e.g., chemical spill). The NTPSI, ECVAM, and/or JaCVAM
273 also obtained Certificates of Analysis for reference standards, controls and reference substances.

274 Procedures for shipping substances to the participating laboratories were the same regardless of whether
275 NTPSI, ECVAM, or JaCVAM was the responsible party. Substances were packaged so as to minimize
276 damage during transit, and shipped under appropriate storage conditions and according to the appropriate
277 regulatory transportation procedures. The study management maintained certificates of analysis for all
278 test substances. The participating laboratories were notified upon shipment in order to prepare for receipt.
279 The Validation Study Project Manager knew who would accept test substance shipments at each
280 participating laboratory. Information regarding weight or volume and storage conditions for each coded
281 reference substance was also provided to each laboratory well in advance of shipment. The shipment
282 contained instructions for the participating laboratories to:

- 283
- 284 • Contact the NTPSI and the Validation Study Project Manager upon receipt of test substances
 - 285 • Contact the Validation Study Project Manager if test facility personnel opened the health and
286 safety packet at any time, for any reason, during the study
- 287

Do Not Cite, Quote, or Distribute

- 287 EPA US. 2009. Endocrine Disruptor Screening Program Test Guidelines: OPPTS 890.1300 -
288 Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa-9903)). EPA 740-C-09-
289 006. Washington, DC:U.S. Environmental Protection Agency. Available:
290 <http://www.regulations.gov/search/Regs/contentStreamer?objectId=0900006480a41cfa&disposit>
291 [ion=attachment&contentType=pdf](http://www.regulations.gov/search/Regs/contentStreamer?objectId=0900006480a41cfa&disposit)
- 292 ICCVAM. 2002a. Background Review Document: Current Status of Test Methods for Detecting
293 Endocrine Disruptors: In Vitro Estrogen Receptor Binding Assays.National Institute of
294 Environmental Health Sciences. Available:
295 http://iccvam.niehs.nih.gov/docs/endo_docs/final1002/erbnbrd/ERBd034504.pdf
- 296 ICCVAM. 2002b. Background Review Document. Current Status of Test Methods for Detecting
297 Endocrine Disruptors: In Vitro Androgen Receptor Binding Assays
298 Background Review Document. Current Status of Test Methods for Detecting Endocrine
299 Disruptors: In Vitro Androgen Receptor Transcriptional Activation Assays.National Institute of
300 Environmental Health Sciences. Available:
301 http://iccvam.niehs.nih.gov/docs/endo_docs/final1002/arbndbrd/ARBd034506.pdf
302 http://iccvam.niehs.nih.gov/docs/endo_docs/final1002/arta_brd/ARTA034507.pdf
- 303 ICCVAM. 2002c. Background Review Document. Current Status of Test Methods for Detecting
304 Endocrine Disruptors: In Vitro Androgen Receptor Transcriptional Activation Assays.National
305 Institute of Environmental Health Sciences. Available:
306 http://iccvam.niehs.nih.gov/docs/endo_docs/final1002/arta_brd/ARTA034507.pdf
- 307 ICCVAM. 2002d. Background Review Document. Current Status of Test Methods for Detecting
308 Endocrine Disruptors: In Vitro Estrogen Receptor Transcriptional Activation Assays.National
309 Institute of Environmental Health Sciences. Available:
310 http://iccvam.niehs.nih.gov/docs/endo_docs/final1002/erta_brd/ERTA034505.pdf
- 311 ICCVAM. 2003. ICCVAM Evaluation of In Vitro Test Methods For Detecting Potential
312 Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional
313 Activation Assays.National Institute of Environmental Health Sciences. Available:
314 http://iccvam.niehs.nih.gov/docs/endo_docs/edfinalrpt0503/edfinrpt.pdf
- 315 ICCVAM. 2006. Finalized Addendum to ICCVAM Evaluation of In Vitro Test Methods for
316 Detecting Potential Endocrine Disruptors.National Institute of Environmental Health Sciences.
317 Available: http://iccvam.niehs.nih.gov/docs/endo_docs/EDAddendFinal.pdf
- 318 Kanno J, Onyon L, Peddada S, Ashby J, Jacob E, Owens JW. 2003a. The OECD program to
319 validate the rat uterotrophic bioassay. Phase 2: Coded single-dose studies. Environmental Health
320 Perspectives 111(12): 1550-1558.
- 321 Kanno J, Onyon L, Peddada S, Ashby J, Jacob E, Owens JW. 2003b. The OECD program to
322 validate the rat uterotrophic bioassay. Phase 2: Dose-response studies. Environmental Health
323 Perspectives 111(12): 1530-1549.
- 324 OECD. 2007. Test No. 440. Uterotrophic Bioassay in Rodents: A short-term screening test for
325 oestrogenic properties [adopted 16 October 2007]. In: OECD Guidelines for the Testing of

Do Not Cite, Quote, or Distribute

- 326 Chemicals, Section 4: Health Effects OECD Publishing. Available:
327 <http://dx.doi.org/10.1787/9789264067417-en> [accessed 30 July 2010].
- 328 OECD. 2009a. OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects OECD
329 Publishing. Available
330 <http://www.oecdbookshop.org/oecd/display.asp?CID=sourceoecd&LANG=en&SF1=DI&ST1=5>
331 KSB8GXKF3WC
- 332 OECD. 2009b. Test No. 455. Stably Transfected Human Estrogen Receptor- α Transcriptional
333 Activation Assay for Detection of Estrogenic Agonist-Activity of Chemicals [adopted 7
334 September 2009]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health
335 Effects OECD Publishing. Available: <http://dx.doi.org/10.1787/9789264076372-en> [accessed 30
336 July 2010].
- 337 Owens JW, Ashby J. 2002. Critical review and evaluation of the uterotrophic bioassay for the
338 identification of possible estrogen agonists and antagonists: In support of the validation of the
339 OECD uterotrophic protocols for the laboratory rodent. *Crit Rev Toxicol* 32(6): 445-520.
- 340 Owens JW, Koeter HBWM. 2003. The OECD program to validate the rat uterotrophic bioassay:
341 An overview. *Environmental Health Perspectives* 111(12): 1527-1529.
- 342 Takeyoshi M. 2006. Draft Report of Pre-validation and Inter-laboratory Validation For Stably
343 Transfected Transcriptional Activation (TA) Assay to Detect Estrogenic Activity - The Human
344 Estrogen Receptor Alpha Mediated Reporter Gene Assay Using hER-HeLa-9903 Cell Line:
345 Chemicals Evaluation and Research Institute (CERI), Japan.
346
347