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Background Review Document

Current Status of Test Methods for Detecting Endocrine Disruptors: *In Vitro* Estrogen Receptor Binding Assays

National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
Department of Health and Human Services

THE INTERAGENCY COORDINATING COMMITTEE ON THE VALIDATION OF ALTERNATIVE METHODS
and
THE NTP INTERAGENCY CENTER FOR THE EVALUATION OF ALTERNATIVE TOXICOLOGICAL METHODS

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) was established in 1997 by the Director of the National Institute of Environmental Health Sciences (NIEHS) to implement NIEHS directives in Public Law 103-43. P.L. 103-43 directed NIEHS to develop and validate new test methods, and to establish criteria and processes for the validation and regulatory acceptance of toxicological testing methods. P. L. 106-545, the ICCVAM Authorization Act of 2000, establishes ICCVAM as a permanent committee. The Committee is composed of representatives from 15 Federal regulatory and research agencies that generate, use, or provide information from toxicity test methods for risk assessment purposes. P.L. 106-545 directs ICCVAM to coordinate technical reviews and evaluations of new, revised, and alternative test methods of interagency interest. ICCVAM also coordinates cross-agency issues relating to development, validation, acceptance, and national/international harmonization of toxicological test methods.

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) was established in 1998 to provide operational support for the ICCVAM and to collaborate with the ICCVAM to carry out committee-related activities such as test method peer reviews and workshops. NICEATM and ICCVAM coordinate the scientific review of the validation status of proposed methods and provide recommendations regarding their usefulness to appropriate agencies. NICEATM and ICCVAM seek to promote the validation and regulatory acceptance of toxicological test methods that will enhance agencies' abilities to assess risks and make decisions, and that will refine, reduce, and replace animal use. The ultimate goal is to improve public health by gaining the regulatory acceptance of new scientifically valid test methods that are more predictive of human and ecological effects than currently available methods.

Additional Information

Additional information can be found at the ICCVAM/NICEATM Website: *http://iccvam.niehs.nih.gov* and in the publication: *Validation and Regulatory Acceptance of Toxicological Test Methods, a Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods* (NIH Publication No. 97-3981), or you may contact the Center at telephone 919-541-3398, or by e-mail at *iccvam@niehs.nih.gov*. Specific questions about ICCVAM and the Center can be directed to the Director of NICEATM:

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Department of Transportation
Environmental Protection Agency
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On the Cover

The ICCVAM/NICEATM graphic symbolizes the important role of new and alternative toxicological methods in protecting and advancing the health of people, animals, and our environment.

NOTICE TO READER

This Background Review Document contains data, a proposed list of substances, and minimum procedural standards that were reviewed by an independent Expert Panel in May 2002.

The reader is referred to the final report entitled, "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) for the final ICCVAM recommended substances and minimum procedural standards.

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Current Status of Test Methods for Detecting Endocrine Disruptors: *In Vitro* Estrogen Receptor Binding Assays

Background Review Document

Prepared for
The National Toxicology Program (NTP)
Interagency Center for the Evaluation of Alternative Toxicological Methods
(NICEATM)

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A	Observed anisotropy
A_o	Anistropy at 0% inhibition
A_{100}	Anistropy at 100% inhibition
A_b	Anisotropy values of bound ligand
A_f	Anisotropy values of free ligand
ACC	American Chemistry Council
AR	Androgen receptor
B_{max}	The number of binding sites in a cytosolic preparation
BRD	Background Review Document
CASRN	Chemical Abstracts Service Registry Number
CMA	Chemical Manufacturers Association
cDNA	Complementary DNA
Ci	Curies
DDT	Dichlorodiphenyltrichloroethane
def	Ligand binding domain of the estrogen receptor protein
DES	Diethylstilbestrol
DMSO	Dimethyl sulfoxide
DPM	Disintegrations per minute
ED	Endocrine disruptor
EDSP	Endocrine Disruptor Screening Program
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee
EDTA	Ethylenediamine tetraacetic acid
EPA	(U.S.) Environmental Protection Agency
ER	Estrogen receptor
ERα	Estrogen receptor alpha
ERβ	Estrogen receptor beta
ER_b	Bound estrogen receptor
ER_f	Free estrogen receptor

ER_t	Total estrogen receptor
ERE	Estrogen response element
F_b	Fraction of ligand bound
FDA	(U.S.) Food and Drug Administration
FES1	Fluormone intrinsically fluorescent non-steroidal estrogen
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FFDCA	Federal Food, Drug, and Cosmetic Act
fmol	Femtomole
FP	Fluorescent polarization
FQPA	Food Quality Protection Act
g	Gravity
GLM	General linear models
GLP	Good Laboratory Practices
GST	Glutathione-*S*-transferase
GST-aERdef	Glutathione-*S*-transferase fusion protein containing the ligand binding domain of the lizard (anole) estrogen receptor
GST-cERdef	Glutathione-*S*-transferase fusion protein containing the ligand binding domain of the chicken estrogen receptor
GST-ERdef	Glutathione-*S*-transferase fusion protein containing the ligand binding domain of the estrogen receptor
GST-hERα def	Glutathione-*S*-transferase fusion protein containing the ligand binding domain of human estrogen receptor alpha
GST-mERαdef	Glutathione-*S*-transferase fusion protein containing the ligand binding domain of mouse estrogen receptor alpha
GST-rtERdef	Glutathione-*S*-transferase fusion protein containing the ligand binding domain of the rainbow trout estrogen receptor
HAP	Hydroxyapatite
HDT	Highest dose tested
hERα	Human estrogen receptor alpha
hERα-FP	Human estrogen receptor alpha as measured by FP
hERβ	Human estrogen receptor beta

HPTE	2,2-Bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane
$I_{\%}$	Percent inhibition
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
IC_{50}	Concentration of test substance (inhibitor) that displaces 50% of the reference estrogen from the receptor in a competitive binding assay
kDa	Kilodaltons
K_d	Dissociation or binding constant
K_i	Equilibrium dissociation constant of a receptor-ligand complex
L_b	Bound ligand
ln	Natural log
L_t	Total ligand
µg	Microgram
µL	Microliter
µM	Micromolar
M	Molar
MCF-7	Cell line derived from a human mammary adenocarcinoma
mL	Milliliters
mM	Millimolar
mmol	Millimoles
MUC	Mouse uterine cytosol
NAS	(U.S.) National Academy of Sciences
NCTR	National Center for Toxicological Research
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
nM	Nanomolar
NSB	Nonspecific binding
NTP	(U.S.) National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCB	Polychlorinated biphenyl

pM	Picomolar
pmol	Picomoles
ppm	Parts per million
QA	Quality assurance
QSAR	Quantitative structure activity relationship
r_I	Intra-class correlation
RBA	Relative binding affinity
RBC	Rabbit uterine cytosol
rERβ	Rat estrogen receptor beta
RUC	Rat uterine cytosol
SAB	Science Advisory Board
SAP	Scientific Advisory Panel
SDWA	Safe Drinking Water Act
SE	Standard error
SERM	Selective Estrogen Receptor Modulator
TEDG	Buffer containing Tris, EDTA, dithiothreitol, and glycerol buffer; used in the isolation of ER from animal tissues
Tris	Tris(hydroxymethyl)aminomethane
TSCA	Toxic Substances Control Act
WWF	World Wildlife Fund

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PREFACE

The Food Quality Protection Act and Amendments to the Safe Drinking Water Act in 1996 directed the U.S. Environmental Protection Agency (U.S. EPA) to develop and validate a screening and testing program, to determine whether certain substances may have hormonal effects in humans. In response, the U.S. EPA developed an Endocrine Disruptor Screening Program (EDSP). The U.S. EPA is currently evaluating the scientific validity of screening and testing methods proposed for incorporation into the EDSP. *In vitro* estrogen receptor (ER) and androgen receptor (AR) assays have been proposed as possible components of the EDSP Tier 1 screening battery. The U.S. EPA asked the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to evaluate the validation status of these *in vitro* assays. ICCVAM, which is charged with coordinating the technical evaluations of new, revised, and alternative test methods, agreed to evaluate the assays based on their potential interagency applicability and public health significance.

In order to assess the current validation status of these *in vitro* methods, it was first necessary to compile all of the available data and information for existing assays. The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), which provides operational support for the ICCVAM, subsequently arranged for preparation of this Background Review Document (BRD) by its support contractor, Integrated Laboratory Systems, Inc. (ILS) with financial support from the U.S. EPA. This BRD reviews available data and procedures for existing *in vitro* ER binding assays and is organized according to published guidelines for submission of test methods to ICCVAM (ICCVAM, 1999). Separate BRDs have also been prepared for *in vitro* AR binding assays, *in vitro* ER transcriptional activation assays, and *in vitro* AR transcriptional activation assays.

As part of the ICCVAM evaluation, the U.S. EPA also asked for development of minimum performance criteria that could be used to define an acceptable *in vitro* ER binding assay. It was envisioned that these criteria would be based on the performance of existing standardized *in vitro* ER binding assays. The minimum performance criteria could be used to assess the acceptability of other new or revised assays proposed in the future. However, a comprehensive review determined that there were no standardized *in vitro* ER binding assays with adequate validation

data that could serve as the basis for establishing these performance criteria. An independent Expert Panel (Panel) was therefore convened to assess the status of existing *in vitro* ER binding assays and to develop recommendations for standardized assays and validation studies that should be conducted. After adequate validation studies have been completed on one or more standardized ER binding assays, an independent Peer Review Panel will be convened to evaluate the validated assay(s) and to recommend minimum performance criteria for *in vitro* ER binding assays.

This BRD reviews available *in vitro* ER binding assays and presents the data available for substances evaluated in these assays. The relative performance of various types of *in vitro* ER binding assays is compared using this existing data, which was very limited for some of the assays. Based on the comparative performance and advantages and disadvantages of each type of assay, several assays are proposed as priority candidates for standardization and future validation. In addition, minimum procedural standards that should be used for *in vitro* ER binding assays are proposed. These standards include elements such as dose selection criteria, minimum number of replicates, appropriate positive and negative controls, criteria for an acceptable test run, and proficiency standards for participating laboratories. Finally, the BRD proposes a list of substances recommended for the validation of *in vitro* ER binding screening assays.

An Expert Panel was convened in a public meeting on May 21-22, 2002, to review the information and proposals provided in this BRD, and to develop conclusions and recommendations on the following:

- Specific assays 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 assays recommended for validation studies.
- The adequacy and appropriateness of substances proposed for validation studies.

The Expert Panel meeting was announced to the public in a *Federal Register* notice (Vol. 67, No. 66, pp. 16415-16416, April 5, 2002; also available on the internet at: http://iccvam.niehs.nih.gov/docs/FR/6716415.pdf)

An ICCVAM Endocrine Disruptor Working Group (EDWG) was organized to coordinate the technical evaluation of *in vitro* endocrine disruptor screening methods. The EDWG is co-chaired by Drs. David Hattan and Marilyn Wind, and consists of knowledgeable scientists from ICCVAM agencies. The EDWG's functions include identification and recommendation of experts for the Expert and Peer Review Panels, the review of test method BRDs for completeness, preparation of questions for the Expert and Peer Review Panels, and development of draft ICCVAM test recommendations based on Panel evaluations. Final ICCVAM test recommendations are then forwarded from the ICCVAM to Federal agencies for their consideration.

In August 2002, the draft of this BRD was revised to address corrections and omissions noted by the Expert Panel and published as a final version. The final report of the Expert Panel and a proposed list of substances for validation studies of *in vitro* ER and AR methods was published and made available to the public for comment as announced in a *Federal Register* notice (Vol. 67, No. 204, pp. 64902-64903, October 22, 2002; available at http://iccvam.niehs.nih.gov/docs/FR/6764902.htm). A final ICCVAM Test Method Evaluation report was published in May 2003. This report includes ICCVAM recommendations, the final Expert Panel report, a recommended list of substances for validation studies, and public comments. The report will be forwarded to federal agencies for their consideration and made available to the public.

The efforts of the many individuals who contributed to the preparation, review, and revision of this BRD are gratefully acknowledged. These include Barbara Shane, Christina Inhof, Errol Zeiger, Raymond Tice, Bradley Blackard, Steven Myers, and Linda Litchfield, from ILS, Inc. who prepared the BRD. The suggestions and advice from the ICCVAM EDWG members and Co-Chairs on early drafts and subsequent versions were invaluable, as were the comments from *ad hoc* reviewers on the final draft. Additional comments and suggestions for improvement of this and future test method documents are welcome at any time.

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EXECUTIVE SUMMARY

The objectives of this BRD are to: (1) provide comprehensive summaries of the published and publicly available unpublished data on the scientific basis and performance of *in vitro* assays used to test substances for their ability to bind to the estrogen receptor (ER); (2) assess the *in vitro* ER binding assays considered for their effectiveness in identifying endocrine-active substances; (3) identify and prioritize *in vitro* ER binding assays that might be considered for incorporation into future testing programs for validation; 4) develop minimum performance criteria by which to judge the effectiveness of proposed *in vitro* ER binding assays; and (5) generate a list of recommended substances to be used in validation efforts.

The data summarized in this BRD are based primarily on information obtained from the peer-reviewed scientific literature. An online literature search was conducted to retrieve records on publications reporting on the testing of substances for their endocrine disrupting effects *in vitro*. Of the 459 records obtained from the initial search, 260 of these citations contained information on ER binding. Data from 72 of these publications were included in this BRD. Some of the peer-reviewed publications that contained ER binding data were not abstracted for inclusion in this BRD because the studies lacked the appropriate details or contained data from unique procedures or substances that were not clearly identified.

Data were abstracted from 14 different ER binding assays. These assays used ER derived from uterine cytosol from the mouse (MUC), rat (RUC), and rabbit (RBC); from MCF-7 cells and MCF-7 cytosol; from human cDNA clones of the two human ER subtypes, ERα and ERβ (hERα and hERβ), and from cDNA clones of rat ERβ (rERβ). Fusion proteins in which glutathione-*S*-transferase (GST) was fused with the *def* domains of the human ERα (GST-hERα), and the ER from mice (GST-mER), chicken (GST-cER), anole (GST-aER), and rainbow trout (GST-rtER) served as the ER source for five assays. All of the assays except one measured the competitive displacement of radiolabeled ([^{3}H] or [^{131}I]) 17β-estradiol from the ER. One assay, designated as hERα-FP, measured the displacement of a fluorescently-labeled estrogen ligand by the test substances using fluorescent polarization (FP).

The majority of the 638 substances tested for *in vitro* ER binding could be classified into one of the following chemical classes: polychlorinated biphenyls, phenolic and non-phenolic steroids, triphenylethylenes, organochlorines, polycyclic aromatic hydrocarbons, stilbenes, phenols, and bisphenols. Only 50% of the substances could be assigned to a product class, the most common of which were pharmaceuticals, pesticides, chemical intermediates, dielectric fluids or their components, natural products (including several phytoestrogens), and plasticizers.

More than half the substances (376; 59%) were tested in the RUC assay, and 133 (21%) of the substances were tested in the next most frequently used assay, hERα. For five of the 14 assays (hERα-FP, RBC, rERβ, GST-mERαdef, GST-cERdef), published data were located on fewer than 50 substances per assay. Only 15 (2.4%) of the substances had been tested in 10 or more assays, and of these, only four (0.6%) had been tested in all 14 assays; in contrast, 403 (63.2%) of substances had been tested in one assay only.

The majority of the publications reported the data as IC_{50} values or relative binding affinities (RBA), that is, the ratio of the IC_{50} of the reference estrogen, 17β-estradiol, divided by the IC_{50} of the test substance and multiplied by 100.

Although a large number of substances have been tested in these *in vitro* ER binding assays, relatively few have been tested more than once in the same assay or in multiple assays. Furthermore, because the primary focus of many of the studies reviewed in this BRD was on understanding the mechanisms of ER binding, and not on identifying substances with ER-binding activity, much of the published data are of limited value for the analysis of performance or reliability of these assays.

To assess comparative assay performance, a quantitative assessment was conducted using the available IC_{50} and RBA data after log normal transformation of the data to reduce possible skewness. In this analysis, only positive responses were considered (i.e., discordant positive and negative results for the same substance in the same assay were not taken into account). The quantitative assessment of the data showed that the effect of substances on the variation in RBA and IC_{50} values was much greater than the effect of assay type, and that there were no significant

differences in performance among the different *in vitro* ER binding assays. This quantitative assessment was limited by the lack of multiple test data within an assay for most of the substances, and by the lack of data across all assays for many substances.

A qualitative assessment of the IC_{50} data, which considered both negative and positive results, was performed also. This assessment considered whether RBA values (single or median) obtained for substances tested in each of 13 assays were within the same log range as the corresponding values obtained for the same substances when tested in the RUC assay. Based on this qualitative approach, the hERα, hERα-FP, hERβ, rERβ, GST-rtERdef, and MUC assays performed better than the RUC assay; the MCF-7 cytosol assay performed about as well as the RUC assay; and the remaining GST-ERdef assays, the MCF-7 cell assay, and the RBC assay did not perform as well as the RUC assay.

To assess assay reliability, a quantitative assessment was conducted using log normal transformed IC_{50} and RBA data. Again, only positive responses were considered. An analysis of the variances for the RBA values of 12 substances that had been tested in at least nine of the 14 *in vitro* ER binding assays suggested that there were no statistically significant differences in the reliability of the assays as performed by different laboratories. A comparison of the variability in RBA and IC_{50} values across assays, ignoring substance effects, suggested that the RUC and hERβ assays were the most consistent, and that the RBC assay was the least consistent among the 14 assays evaluated. An analysis of the variability in the IC_{50} for the reference control chemical, 17β-estradiol, indicated that the most consistent results were obtained with the hERα-FP assay, while the MUC, RUC, and hERα assays exhibited somewhat greater, but comparable, variances. The low variability associated with the hERα-FP assay, however, might be a reflection of the small number of laboratories that have reported IC_{50} values using this method.

Generally, the databases for all the *in vitro* ER binding assays considered in this BRD are too limited to draw any sound conclusions regarding their performance and reliability. However, based on general principles, recommendations were made in regard to the use of *in vitro* ER binding assays as a component of a Tier 1 endocrine disruptor screening battery:

- Based on a consideration of such factors as relative performance, elimination of animal use, the use of the ER from the species of interest, and the use of alternatives to radioactive substances, the hERα, hERα-FP, and hERβ assays should have the highest priority for validation as screening assays for human health-related issues, while the GST-rtERdef assay might be preferred when screening for substances that pose a hazard to wildlife.
- In conducting future validation studies with these assays, the RUC assay should be used as the reference test method. The RUC assay is currently undergoing validation efforts sponsored by the U.S. EPA and the resulting performance and reliability information could be used to establish minimal performance standards for other assays.
- Formal validation studies should be conducted using appropriate substances covering the range of expected RBA values to adequately demonstrate the performance characteristics of the *in vitro* ER binding assays recommended as possible screening assays.
- There is little information about the ER binding activity of metabolites of xenobiotics and it is not clear whether metabolic activation needs to be included in an *in vitro* ER binding test method used as a screening assay. This issue should be considered prior to the implementation of future validation studies.

An important step towards acceptance of an *in vitro* ER binding assay into a regulatory screening program is production of high quality data. To achieve this goal, it is recommended that any future prevalidation and validation studies on *in vitro* ER binding assays be conducted with coded substances and in compliance with GLP guidelines. Ideally, if multiple laboratories are involved in the validation study, the substances should be obtained from a common source and distributed from a central location.

In conducting these validation studies, all of the original data and documentation supporting the validation of a test method must be carefully documented, and include detailed protocols under which the data were produced.

If an assay chosen for validation requires the use of animals, the studies should be conducted to minimize the number of animals used, and animal pain and distress. Adoption of one of the

assays using purified or semi-purified receptors, or glutathione fusion proteins would eliminate the use of animals for *in vitro* ER binding experiments.

Since there are no published guidelines for conducting *in vitro* ER binding studies, and no formal validation studies have been performed to assess the reliability or performance of ER binding assays, the U.S. EPA requested that minimum procedural standards based on a comparative evaluation of *in vitro* ER binding assays be provided. In addition it was requested that a recommended list of test substances be provided for use in validation studies.

The minimum procedural standards include methods for determining the K_d of the reference estrogen, methods for test substance preparation, the concentration range of the test substance (including the limit dose), the use of negative and positive controls, the number of replicates per test substance concentration, dose spacing, assay acceptance criteria, data analysis, evaluation and interpretation of results, minimal information to include in the test report, and the need for replicate studies.

Based on a RUC protocol provided by the U.S. EPA, a suggested general protocol for measuring ER binding using the RUC assay was developed as a potential resource for scientists interested in developing their own laboratory specific protocol. This general RUC protocol incorporated the recommended minimum procedural standards. Various aspects of the assay performance, including preparation of the ER, reagents and solutions, measurement of ER binding, evaluation of the data, and test report guidance are described.

In the development of a list of reference substances for use in validation studies, consideration was given to the number of times the substance had been tested in the RUC assay, the median RBA value of the substance in the RUC assay, and the extent of concordance of the median RBA value in the RUC assay with values obtained for the same substance in other *in vitro* ER binding assays. The substances were then sorted according to their median RBA values, which ranged over seven orders of magnitude. Five substances were selected for each RBA category ($\geq$10, <10-1; <1-0.1; <0.01-0.1, <0.01-0.001; <0.001) and three for a negative category group. Weakly-binding substances (RBA values <0.001) were difficult to identify because they were

not consistently positive in tests within an assay or among different assays. Substances were classified as "negative" for ER binding based on the lack of a positive response in multiple assays when tested at concentrations of at least 1 mM. When possible, representatives of the most common classes of substances were included in each RBA category.

It is anticipated that this BRD and the guidance it provides will help to stimulate validation efforts for *in vitro* ER binding assays.

1.0 INTRODUCTION AND RATIONALE FOR THE USE OF *IN VITRO* ER BINDING ASSAYS

1.1 Introduction

1.1.1 Historical Background of *In Vitro* Endocrine Disruptor Assays and Rationale for Their Development

It is well known that small disturbances in endocrine function, especially during highly sensitive stages of the life cycle (e.g., fetal and prepubertal development), can lead to significant and lasting effects on the exposed organism (Kavlock et al., 1996; U.S. EPA, 1997; NAS, 1999). In recent years, evidence has been accumulating to suggest that exposure to natural and anthropogenic substances in the environment may adversely affect the endocrine and reproductive systems of mammals, fish, reptiles, amphibians, and birds. Substances that cause such effects are classified as "endocrine disruptors". Disruption of the endocrine system has been demonstrated in laboratory animals and documented in wildlife (Ankley et al., 1998). For example, male fish caught in rivers in many regions of the United States have high levels of vitellogenin, a female-specific protein (Purdom et al., 1994; Folmar et al., 1996), and female mosquitofish living in streams in which pulp mill effluents containing steroidal substances have been discharged possess male gonadal structures (Bortone et al., 1989). The degree to which humans are affected by endocrine disruptors is unknown, although there are reports that these substances might be contributing to increasing incidences of breast, prostate, and testicular cancers (Glass and Hoover, 1990; Adami et al., 1994; Toppari et al., 1996) and to precocious puberty, hypospadias, and decreased sperm counts (Carlsen et al., 1992; Sharpe and Skakkabaek, 1993). However, other investigators have concluded that there is no evidence for endocrine disrupting effects in humans (Safe, 2000; Barlow et al., 1999).

In 1996, the U.S. Congress responded to societal concerns by passing legislation requiring the U.S. EPA to develop a screening and testing program, using appropriately validated test methods, to detect potential endocrine disruptors in pesticide formulations (the Food Quality Protection Act; FQPA) (P.L. 104-170), and in drinking water (the 1996 amendments to the Safe Drinking Water Act; SDWA) (P.L. 104-182). As a result of these mandates, the U.S. EPA formed the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) to provide advice on how to best design a screening and testing program for identifying endocrine

disruptors. In August 1998, EDSTAC issued a report recommending that the U. S. EPA evaluate both human and ecological (wildlife) effects; examine effects to estrogen, androgen, and thyroid hormone-related processes; and test both individual substances and common mixtures (U.S. EPA, 1998a). In December 1998, based on these recommendations, the U.S. EPA proposed the EDSP (U.S. EPA, 1998b). In 1999, the EDSP and its proposed approach to screening for endocrine disruptors were endorsed by the U.S. EPA Science Advisory Board (SAB) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP), which also made a number of recommendations concerning the proposed approach (U.S. EPA, 1999).

The EDSP proposes a two-tiered approach for screening and testing. Tier 1 is comprised of *in vitro* and *in vivo* assays and is designed as a screening battery to detect substances capable of interacting with the estrogen, androgen, or thyroid hormone systems. Tier 2 is comprised of *in vivo* assays only and is designed as a testing battery to (1) determine whether an endocrine-active substance (identified in Tier 1 or through other processes) causes adverse effects in animals; (2) identify the adverse effects; and (3) establish a quantitative relationship between the dose and the adverse effect (U.S. EPA, 2000).

The EDSP's proposed Tier 1 screening battery includes the following assays:
In vitro assays:

- ER binding/transcriptional activation (TA) assay
- AR binding/TA assay
- Steroidogenesis assay with minced testis

In vivo assays:

- Rodent 3-day uterotrophic assay (subcutaneous dosing)
- Rodent 20-day pubertal female assay with enhanced thyroid endpoints
- Rodent 5-7 day Hershberger assay
- Frog metamorphosis assay
- Fish gonadal recrudescence assay

The alternative Tier 1 assays include:

- Placental aromatase assay (*in vitro*)
- Modified rodent 3-day uterotrophic assay with intraperitoneal dosing (*in vivo*)
- Rodent 14-day intact adult male assay with thyroid endpoints (*in vivo*)
- Rodent 20-day thyroid/pubertal male assay (*in vivo*)

According to the EDSP, the Tier 1 assays should:

- Detect all known modes of action for the endocrine endpoints of concern;
- Maximize sensitivity to minimize false negatives, while permitting a to-be-determined level of false positives;
- Include a sufficient range of taxonomic groups among the test organisms to reduce the likelihood that important pathways for metabolic activation or detoxification of the test substances are not overlooked; and
- Incorporate sufficient diversity among the endpoints and assays to permit conclusions based on weight-of-evidence considerations.

The proposed Tier 2 testing battery includes the following *in vivo* assays:

- Two-generation mammalian reproductive toxicity assay
- Avian reproduction assay
- Fish reproduction assay
- Amphibian reproduction and developmental toxicity assay
- Invertebrate reproduction

The alternative Tier 2 assays include:

- Alternative mammalian reproductive test
- One-generation mammalian reproduction toxicity test

According to the EDSP, the Tier 2 assays should:

- Encompass critical life stages and processes in mammals (equivalent to humans), fish, and wildlife;

- Encompass a broad range of doses and the administration of the test substance by a relevant route of exposure; and
- Provide a comprehensive profile of biological consequences of substance exposure and relate such results to the causal dose and exposure.

Two proposed *in vitro* components of the Tier 1 screening battery are ER binding/TA assays, and AR binding/TA assays. The primary rationale for inclusion of *in vitro* assays in the EDSP Tier 1 screen is that they:

- Are suitable for large-scale screening;
- Are based on well-elucidated mechanisms of action; and
- Measure specific endpoints.

The Tier 1 assays are informative with regard to the mechanism of action of the presumptive endocrine disruptor and provide guidance for prioritization for further testing. Due to their sensitivity, these *in vitro* tests should permit the identification of an active substance(s) within a complex mixture. TA assays have an advantage over binding assays because they can measure if there is a biological response to receptor binding (i.e., RNA transcription) and thus, unlike binding assays, can distinguish between an agonist (a substance that mimics the action of endogenous hormones) and an antagonist (a substance that binds to a receptor without initiating a biological response, blocking the action of endogenous hormones) (U.S. EPA, 1998b). However, it needs to be emphasized that these *in vitro* assays cannot be used to predict the risk for an adverse health effect in humans or wildlife. Binding assays only measure the physical binding of a substance to the receptor while TA assays infer, but do not prove, that an adverse health outcome can occur *in vivo*.

As part of the validation process for the proposed EDSP assays, the U.S. EPA is supporting an effort to prepare a series of BRDs on the Tier 1 *in vitro* ER binding, AR binding, ER TA, and AR TA screening assays. Other EDSP-proposed assays will be evaluated through other organizations (e.g., the U.S. EPA and the Organisation for Economic Co-operation and Development [OECD]). The objectives of each BRD are to:

- Provide a comprehensive summary of the available published and publicly available unpublished data on the scientific basis and performance of the identified assays;
- Identify available assays that might be considered for incorporation into the EDSP;
- Assess their effectiveness for identifying endocrine-active substances;
- Develop minimal procedural standards for acceptable ER and AR binding and TA assays; and
- Provide a list of candidate substances for future validation studies.

1.1.2 Prior or Proposed Peer Reviews of *In Vitro* ER Binding Assays

Although there has been extensive research conducted in the past few years to develop new and improved *in vitro* assays to identify substances with ER binding and transcriptional activity, there have been no formal peer reviews of the validation status of such assays. This BRD has been prepared for an upcoming ICCVAM expert evaluation of the validation status *in vitro* ER binding assays, in concert with reviews of ER TA assays and *in vitro* AR binding and TA assays.

1.2 Scientific Basis for the Proposed Tier 1 *In Vitro* ER Binding Assays

1.2.1 Purpose for Using *In Vitro* ER Binding Assays

In vitro ER binding assays are designed to identify substances (ligands) that bind to the ER and that might act as an estrogenic agonist and cause estrogenic effects, or interfere with normal estrogen activity *in vivo* by acting as an antagonist. The assays can be divided into two mechanistic categories: those that measure binding to the receptor and those that measure transcriptional activation subsequent to binding to the receptor. Although receptor binding assays detect both agonists and antagonists, they do not distinguish between the two. In contrast, TA assays can be designed to distinguish between agonists and antagonists.

Binding of the natural ligand, 17β-estradiol, to the ER is a prerequisite for the induction of many subsequent estrogenic effects, such as induction of cell proliferation in the uterus, and maintenance of bone and the cardiovascular system. The binding affinity of a xenobiotic substance for the ER determines how well it will compete with 17β-estradiol. *In vitro* ER competitive binding assays are generally performed by quantifying the ability of substances to compete with 17β-estradiol for binding. However, ER binding alone is not sufficient to indicate

or predict subsequent cellular effects. For this reason, *in vitro* ER binding assays will be used in conjunction with other *in vitro* and *in vivo* assays for Tier 1 screening. Results from such assays will be used in a weight-of-evidence approach to select substances for Tier 2 testing.

1.2.2 Development of *In Vitro* ER Binding Assays: Historical Background

The foundation of current *in vitro* assays for detecting the ability of substances to bind to the ER can be traced back to the mid-1960s when the receptor was first isolated. At that time, procedures were developed not only for isolation of the receptor but also for the measurement of 17β-estradiol binding and the competitive binding of other substances to the receptor.

The ER was first identified, isolated, and characterized as a protein by Toft and Gorski (1965; 1966) and Noteboom and Gorski (1965) from the soluble fraction of the rat uterus using radiolabeled 17β-estradiol. In these studies, it was shown that the synthetic estrogen diethylstilbestrol competed with 17β-estradiol for binding, but that the non-estrogenic hormones, testosterone and corticosterone, did not bind the ER, and 17α-estradiol was partially inhibitory. Noteboom and Gorski (1965) also initiated the use of radiolabeled (with tritium, [^{3}H]) 17β-estradiol for measuring receptor binding affinity and showed the response to be stereospecific. These studies were extended by Toft et al. (1967), who showed that a cell-free system derived from rat uterine tissue had the same estrogen-binding properties as were found at physiological concentrations *in vivo*. A Scatchard plot (Scatchard, 1949) was used to determine the dissociation constant of 17β-estradiol for the ER and the number of binding sites in the tissue preparation. The size of the ER was subsequently estimated as 53 kDa. The estimated dissociation constant for 17β-estradiol was $7x10^{-10}$ M. Notides (1970) demonstrated that the 17β-estradiol dissociation constants for receptors isolated from rat uterus and anterior pituitary were similar ($1.55x10^{-9}$ compared to $1.40x10^{-9}$ M) and that the responses of these receptors to estrogenic antagonists were essentially identical.

The translocation of the ER complex from the cytosol into the nucleus and its interaction with chromatin was suggested by the work of Shyamala and Gorski (1968) and Jensen et al. (1968). Gorski et al. (1968) hypothesized that the translocated ER complex had DNA-regulatory activity. Clark and Gorski (1969) used a cell-free system to demonstrate that the ER complex bound

equally well to the "nuclear pellet" derived from the uterus, which contains ER, to the kidney, which lacks ER, and to glass pellets. This observation demonstrated that there are no specific nuclear (as opposed to DNA) receptors for the complex.

Between 1965 and 1971, a number of *in vitro* methods were developed to measure the binding of 17β-estradiol and other substances to the ER. Hähnel (1971) and Jungblut et al. (1972) evaluated a number of these *in vitro* methods using cytoplasmic ERs isolated from calf uteri and human breast cancer tissue. They concluded that the dextran-coated charcoal, Sephadex chromatography, and agar electrophoresis methods for the separation of the receptor-bound ligand from unbound, radiolabeled 17β-estradiol were suitable for routine use and had equivalent sensitivities. However, Jungblut et al. (1972) concluded that the dextran-charcoal procedure would be the most suitable because its labor, time, and cost requirements were the lowest of the three methods. Hähnel (1971) and Shafie and Brooks (1979) evaluated the effects of other protocol factors on the binding of 17β-estradiol to the ER and the measurement of unbound fraction. The factors evaluated included pH, storage time of the cytosolic preparation, time and temperature of incubation of 17β-estradiol with the ER, 17β-estradiol concentration, sulfhydryl blocking reagents, protein concentration of the cytosol, and the competitive absorption of estrogen to the charcoal. Erdos et al. (1970) developed a hydroxyapatite (HAP)-column binding procedure that was able to distinguish 17β-estradiol binding to high-affinity versus low-affinity receptor sites.

In the late 1960s and early 1970s, it became apparent that a competitive *in vitro* binding assay would be useful. In such an assay, an ER that contains bound 17β-estradiol is challenged with other substances to determine if these substances alter its binding. One of the earliest studies was performed by Korenman (1970), who measured the comparative binding affinities of natural and synthetic steroids to rabbit cytosol and compared the results with data from an *in vivo* rodent uterotrophic assay. The correlation between the *in vitro* and *in vivo* responses was considered acceptable. The authors noted that the *in vitro* ER binding assay offered many advantages, but that it could not distinguish between agonists and antagonists.

ER binding assays are most often conducted with a cell-free ER preparation obtained from estrogen-responsive tissues or cells. The current procedures used to isolate ER are essentially the same as those used in the late 1960s and early 1970s. Traditional techniques to measure competitive binding are routinely used as well, including the use of dextran-coated charcoal and HAP to separate receptor-bound ligand from free ligand. Although ER binding assays have changed very little over their 30 plus years of use, some of the newer procedures have incorporated more recently developed technology, including the use of recombinant ER proteins in place of ER isolated from tissues or cells (Matthews and Zacharewski, 2000) and measurement of fluorescence polarization (FP) equilibrium binding in place of the measurement of radioactivity (Bolger et al., 1998). The ER binding assays, as currently performed, are described in detail in **Section 2.**

The procedures used to calculate the binding parameters are essentially variations on the method published by Scatchard (1949), who developed models for the binding of small molecules to proteins and for extrapolating binding data. Puca and Bresciani (1968) used Scatchard's procedure to estimate the number of ER binding sites and the 17β-estradiol association constant in isolated calf uterus tissue. In a "Scatchard plot", a straight line indicates that a single class of binding site is present; if competing binding sites are present, the line will deviate from linearity. The intercept on the abscissa indicates the number of binding sites available; the association constant is the ratio of the intercepts on the abscissa and ordinate (Puca and Bresciani, 1968). Scatchard plots are widely used in receptor binding studies.

Baulieu and Raynaud (1970) proposed using an alternative procedure for approximating the binding parameters of small molecules in protein mixtures. They developed a nonlinear function by plotting the log of the bound fraction to the log of the total ligand, and demonstrated that this procedure was able to quantitatively distinguish between specific and nonspecific binding in a tissue extract that contained a mixture of specific and nonspecific receptors.

The ER binding assays measure the affinity of radiolabeled 17β-estradiol for the ER (K_d), the affinity of the unlabeled ligand for the ER (K_i), and the concentration at which the unlabeled ligand displaces half the specific binding of radiolabeled 17β-estradiol to the ER (IC_{50}). The K_d,

which is measured in concentration units, is the equilibrium dissociation constant of the 17β-estradiol-ER complex and represents the concentration of 17β-estradiol that will bind to half the binding sites at equilibrium in the absence of competitors. A low K_d represents high affinity and a high K_d represents low affinity. The K_i is the analogous constant for the unlabeled ligand. The IC_{50} values depend on a number of factors, such as the specific assay system used, binding affinity of the unlabeled ligand for the ER, labeled 17β-estradiol concentration, ER concentration, and experimental conditions (e.g., pH, exposure duration). In *in vitro* ER binding assays, there are substances that, because of biological inactivity, low solubility, or other considerations, do not decrease the binding of labeled, bound 17β-estradiol by at least 50%. The IC_{50} values for these substances are often reported as being greater than the highest concentration tested or they are classified as "non-binders." In this BRD, such substances are classified as negative in the ER binding assay conducted.

Because of the potential for variation in IC_{50} values among ER binding assays or repeats of assays that use different preparations of ER protein, the generally accepted method for presenting and comparing assay results is to compute the relative binding affinity (RBA) of the test substance against a reference estrogen. The RBA is calculated as $IC_{50(\text{reference estrogen})}/IC_{50(\text{test substance})} \times 100$. 17β-Estradiol is generally used as the reference estrogen for calculating the RBA value, but diethylstilbestrol (DES) has also been used. Because RBA values cover approximately eight orders of magnitude and because there is no current guidance as to which levels of activity are biologically meaningful in terms of an adverse health outcome, there is no general agreement regarding the distinction between the values needed to distinguish endocrine disruptors from non-disruptors.

1.2.3 Mechanistic Basis of *In Vitro* ER Binding Assays

The ER is a transcriptional regulatory protein belonging to the nuclear hormone receptor superfamily. The receptor is localized in the soluble nuclear fraction of estrogen target cells and plays a major role in controlling the transcriptional activation and/or repression of estrogen-responsive genes. The ER contains two discrete domains that are necessary for its role as a transcription factor – a ligand-binding domain in the *C*-terminal region and a DNA-binding domain in the *N*-terminal region of the protein. The ligand-binding domain, which is contained

within a wedge-shaped cavity on the receptor, is relatively hydrophobic. This allows the ligand-binding domain to accommodate its endogenous, nonpolar ligand, 17β-estradiol. The DNA-binding domain contains a zinc finger motif found in many DNA-binding proteins (Kumar et al., 1987; Brzozowski et al., 1997).

Recently, a second subtype of the ER, termed ERβ, has been identified (Kuiper et al., 1997). The classical ER is now termed ERα. Many similarities exist between the two subtypes. The DNA-binding domains have about 97% amino acid homology, while the ligand binding domains have about 60% homology (Kuiper and Gustafsson, 1997). Because of these similarities, ERα and ERβ share similar binding kinetics for many but not all of the estrogenic compounds tested with both subtypes. The two subtypes have unique tissue distributions, different physiological roles, and differ in their modes of regulating gene transcription (Kuiper et al., 1998; Gaido et al., 1999).

As the primary receptor for endogenous estrogens that initiate the transcription of messenger RNA and ultimately protein synthesis in estrogen-target cells, the ER plays a pivotal role in the development and maintenance of the female reproductive system. The interaction of estrogens with the ER in a cell initiates a cascade of events, including the dissociation of corepressor proteins from the ER and the induction of significant, conformational changes in the receptor that allow the binding of coactivator proteins. This activated receptor complex binds to specific DNA regulatory sequences of estrogen-responsive genes (estrogen response elements; ERE) that are located upstream from or within the intron regions of the responsive genes. This binding initiates or inhibits the transcription of estrogen-controlled genes, which leads to the initiation or inhibition of cellular processes, respectively, including those necessary for cell proliferation, normal fetal development, or adult homeostasis (Kumar et al., 1987; Brzozowski et al., 1997; Love et al., 2000).

The current hypothesis for ER-mediated endocrine disruption is that certain xenobiotic substances, by virtue of their structure or conformation, bind to the ER and either mimic or block the action of 17β-estradiol. The ER system is a prime candidate for interference by xenobiotic substances because the ligand-binding domain of the ER is much larger than the space occupied

by 17β-estradiol, making the binding site somewhat nonspecific. This nonspecificity has been confirmed by studies demonstrating that a variety of different xenobiotic substances belonging to many structural classes bind to the ER (Blair et al., 2000; Pike et al., 2000; Fang et al., 2001). In addition, some substances, known as selective ER modulators (SERMs), cause the receptor to take on a conformation that is neither fully active nor inactive. SERMs have the ability to act as agonists in some estrogen-responsive tissues and as antagonists in others (McDonnell, 1999).

Potential agonist or antagonist estrogenic activity may be inferred for a substance by its ability to compete with 17β-estradiol for binding to the ER. *In vitro* ER binding assays have been proposed as predictors of estrogen disruption in intact organisms (U.S. EPA, 1997; 1998a,b; 1999). The validity of the binding assay results for this purpose requires a determination that the substance also elicits similar responses in an *in vivo* assay. Such concordance for several substances has been reported by Shelby et al. (1996).

Factors that affect ligand binding to the ER are:

- Affinity for the ER. This affinity depends on the rates of the association and disassociation of the ligand with the receptor. The natural ligand, 17β-estradiol, has a low equilibrium constant because of its rapid association rate and relatively slow disassociation rate. The half-life of the disassociation of 17β-estradiol with the ER in intact rat uterine cells has been reported to be 90 minutes (Kassis et al., 1986).
- Systemic half-life of the ligand. This half-life will depend on its rate of metabolism to an intermediate that binds or does not bind to the receptor, and to the clearance of the ligand and its metabolites from the organism.
- Concentration of the ligand. Weakly binding ligands can produce a biological effect if they are administered at high enough concentrations, and strongly binding ligands would be ineffective if they do not reach estrogen-sensitive tissues.

1.2.4 Relationship of Mechanisms of Action in *In Vitro* ER Binding Assays Compared to the Species of Interest

Although the ER system is highly conserved among vertebrate species, and substances binding to ER derived from one species are expected to bind to the ER from another vertebrate species, the

relative binding affinities of these receptors for the same ligand may be different. Currently, little is known about the comparative binding of ligands to the ER of different species (Ankley et al., 1998). However, the ER from the rainbow trout has been reported to differ both structurally and functionally from its counterpart in mammals (Petit et al., 1995). In this regard, Zacharewski and coworkers (Matthews et al., 2000; Matthews and Zacharewski, 2000) recently showed that polychlorinated biphenyls (PCBs) have more affinity for the binding domain of rainbow trout ER (rtER) than to the binding domains of ERs from human, rat, mouse, or amphibian. Despite these differences and due to a lack of information on interspecies comparisons, the present working hypothesis is that the biological effects in one vertebrate species resulting from exposure to an endocrine disruptor is presumed to occur in other species. This approach is the basis for the use of ER binding as a general screen for estrogenic effects. The most widely used ER binding assays use human or rat ER-containing cells, or cytosolic ER derived from human or rat cells or tissues. Substances that bind the ER from these cells and tissues are presumed to be capable of producing estrogenic effects in multiple species. However, there is insufficient evidence to demonstrate that this extrapolation is appropriate. It is also not known whether differences in ER ligand affinity between species are meaningful with regard to *in vivo* adverse effects.

1.3 Intended Uses of the Proposed *In Vitro* ER Binding Assays

In vitro ER binding assays are proposed components of the EDSP Tier 1 screening battery. The Tier 1 battery is comprised of multiple *in vitro* and *in vivo* assays that assess both receptor- and nonreceptor-mediated mechanisms of action and endpoints. This battery is designed to detect substances that might affect estrogen, androgen, and thyroid hormone systems in multiple species, including humans.

1.3.1 Validation of *In Vitro* Assays

The FQPA requires the U.S. EPA base its endocrine disruptor screening program on validated test systems, and that the assays selected for inclusion in the program be standardized prior to their adoption. The ICCVAM Authorization Act (Sec. 4(c)) mandates that "[e]ach Federal Agency … shall ensure that any new or revised … test method … is determined to be valid for its proposed use prior to requiring, recommending, or encouraging [its use]." (P.L. 106-545,

2000). The validation process will provide data and information that will allow the U.S. EPA to develop guidance on the development and use of functionally equivalent assays and endpoints prior to the implementation of the screening program.

Validation is the process by which the reliability and relevance of an assay for a specific purpose are established (ICCVAM, 1997). Relevance is defined as the extent to which an assay will correctly predict or measure the biological effect of interest (ICCVAM, 1997). For the *in vitro* ER binding assays described in this BRD, relevance is restricted to how well an assay identifies substances that are capable of binding to the ER. The reliability of an assay is defined as its intra- and inter-laboratory reproducibility. Both relevance and reliability should be based on a diverse set of substances representative of the types and range of responses expected to be identified.

The first stage in assessing the validation status of an assay is the preparation of a BRD that presents and examines the available data and information about the assay, including its mechanistic basis, proposed uses, reliability, and performance characteristics (ICCVAM, 1997). This BRD summarizes the available data and information on the various types of *in vitro* ER binding assays that have been commonly used to characterize substances as potential endocrine disruptors. Where appropriate data are available, the qualitative and quantitative performance characteristics of the assays are evaluated, and the reliability of each assay is compared with the reliability of the other assays. These evaluations are used to determine whether a specific assay or assay type (e.g., whole cell, cell cytosol, tissue cytosol, recombinant ER) has been validated sufficiently to allow its recommendation for adoption by the U.S. EPA as an EDSP Tier 1 assay. If there are insufficient data to support the recommendation of an assay, this BRD will aid in identifying which specific assays should undergo further development or validation. The analyses can also be used to identify minimum procedural standards that should be considered for current and future *in vitro* ER binding assays.

1.3.2 Where Can *In Vitro* ER Binding Assays Substitute, Replace, or Complement Existing Methods?

There are no *in vitro* assays for ER binding or TA that are currently accepted by regulatory agencies. The *in vitro* ER binding assays are intended, along with other *in vitro* and *in vivo* tests, to be a component of the proposed EDSP Tier 1 battery for identifying endocrine disruptors.

1.3.3 Similarities and Differences with Currently Used Methods

The measurement of ER binding activity *in vitro* is not currently required for regulatory decision-making. However, there are a number of *in vitro* assays available for measuring receptor binding. These assays are based on the same general principles, but may use different sources of ER and different protocols.

The most frequently used ER binding assays use uterine cytosol from rats and mice as the source of the ER. Cytosol from other sources, such as the breast cancer cell line MCF-7, has also been used. Assays using purified receptor proteins have recently been introduced following the identification of the ERα and ERβ receptors from different tissues. Relative binding of a ligand with the receptor has been measured using either radiolabeled 17β-estradiol or by FP techniques.

1.3.4 Role of *In Vitro* ER Binding Assays in Hazard Assessment

The *in vitro* ER binding assays are proposed as a component of the proposed EDSP Tier 1 screening battery that also includes androgen receptor binding assays, *in vitro* ER and AR TA assays, and *in vivo* assays for endocrine effects in rodents, amphibians, and fish. The EDSTAC committee recognized that TA assays provide more information than binding assays because they measure also the consequences of binding. However, the limited databases at that time did not allow a determination of whether one or the other, or both assays, were preferred for screening (U.S. EPA, 1998a). Subsequently, the EDSP expressed a preference for TA assays over receptor binding assays because these assays can distinguish agonists from antagonists, and can be conducted with and without exogenous metabolic activation (U.S. EPA, 1999).

The assays in the Tier 1 battery have been combined in a manner such that limitations of one assay are complemented by strengths of another. The *in vitro* assays measure the interactions

between the test substance and binding and/or transcriptional activation only, and may therefore produce false positive results that may not occur *in vivo* due to limited absorption, distribution, metabolism, and excretion of the substance. The *in vitro* assays may also produce false negative results due to the absence of active metabolites that are formed *in vivo,* and to endocrine-related effects that are mediated by mechanisms not addressed by the *in vitro* assays.

A positive result in the ER binding assay or in other Tier 1 screening assays would not be sufficient to make the determination that a substance would produce a hormone-related adverse health effect in humans or other species. A weight-of-evidence approach will be used to evaluate the battery of Tier 1 results and to make decisions about whether or not a test substance would be subject to Tier 2 testing (U.S. EPA, 1998b). The Tier 2 assays are all performed *in vivo* and were selected to determine if a substance identified in Tier 1 as a potential endocrine disruptor exhibits endocrine-mediated adverse effects in animals and to identify, characterize, and quantify these effects.

1.3.5 Intended Range of Substances Amenable to *In Vitro* ER Binding Assays and/or Limits of *In Vitro* ER Binding Assays

The range of substances amenable to testing in *in vitro* ER binding assays has yet to be determined and will depend on the outcome of an independent peer review of the assays considered in this BRD. The *in vitro* ER binding assays are intended to be used to test food components and contaminants, as described in the FQPA (P.L. 104-170), and water contaminants, as described in the 1996 Amendments to the SDWA (P.L. 104-182). In addition, the U.S. EPA has authority to test commercial substances regulated by the Toxic Substances Control Act (TSCA, 1976) in the following circumstances: 1) the SDWA provides for testing of TSCA substances present in drinking water; 2) the FQPA amendments and the Federal Food Drug and Cosmetic Act (FFDCA; 21 CFR Ch.9., 1996) provide for testing of "inerts" in pesticide formulations; and 3) the FQPA and FFDCA provide for testing of substances that "act cumulative to a pesticide."

1.4 Search Strategy and Selection of Citations for the *In Vitro* ER Binding BRD

The *in vitro* ER binding data summarized in this BRD are based on information found in the peer-reviewed scientific literature. An online literature search of entries in MEDLINE, CANCERLIT, TOXLINE, AGRICOLA, NIOSHTIC, EMBASE, CABA, BIOSIS, and LifeSci was conducted to retrieve database records on publications reporting on *in vitro* testing of substances for their endocrine disrupting effects. The search was conducted in the database basic index, which includes words in the title and abstract, and indexing words. Specifically, records on estrogen/androgen receptor binding assays and estrogen/androgen TA assays were sought. The search strategy involved the combining of "*vitro*" with alternative terms for estrogens, androgens, receptors, binding, and testing. Each database record included authors, bibliographic citation, and indexing terms. Most records also included abstracts.

Of the 459 records obtained from the initial search conducted on December 12, 2000, 354 contained data from estrogen-related assays and 105 contained data from androgen-related assays. Abstracts of selected titles were reviewed, and the relevant articles were selected and retrieved from the literature for analysis. A database of the literature citations was established using bibliographic database software. Subsequent to the initial search, additional articles with relevant information were identified and retrieved; many of these were identified from the bibliographies of the previously selected articles. Scanning of the literature using *Current Contents* and the British Lending Library's *Table of Contents* continued through the writing of this BRD, and recently published articles were added to the database as they became available. Identification of ER-related publications for data extraction was completed on September 30, 2001.

The most relevant reports were those containing data on substances that have been tested in more than one laboratory using identical or related protocols. Every effort was made to include data from these publications because they provided information that could contribute to the assessment of the performance and reliability of the different assays. Publications containing data for substances that were synthesized specifically for the reported study and were not tested in other laboratories or in other *in vitro* ER binding assays did not contribute to the analysis of the data for performance and reliability. Primarily, these studies compared the binding affinities

of structural and positional isomers of known binding agents (such as 17β-estradiol) that were synthesized specifically for the study and are not available commercially. Data on the ER binding affinity of some of these substances are included in the BRD. Data was not extracted from reports of studies using a unique procedure or from studies that tested obscure or difficult-to-identify substances. Based on these criteria, data from 72 publications was abstracted and included in this BRD.

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2.0 *IN VITRO* ER COMPETITIVE BINDING ASSAY METHODS

2.1 Introduction

The basic procedures to measure test substance binding to the ER were developed between 1965 and the early 1970s (Clark and Gorski, 1969). Cells containing an ER, semi-purified ER, or cytosolic fractions from cells containing an ER (typically from the uterus) are treated with sufficient amounts of radiolabeled reference estrogen (generally 17β-estradiol) to saturate all of the ER binding sites. Following this treatment, the cells, proteins, or cellular extracts are challenged with the test substance, and the amount of radioactive reference estrogen remaining bound to the ER is measured by scintillation counting. The amount of bound radiolabeled reference estrogen is a function of the receptor-binding capacity of the test substance and the test substance concentration. Recently, a technique known as FP, in which a fluorescent estrogen molecule replaces the radiolabeled reference estrogen has been developed. In this assay, changes in the polarization of light are measured rather than scintillation counting of the amount of ER-bound radiolabeled reference estrogen.

Results from these competition assays are expressed as the K_i or as the IC_{50}. The K_i is a function of the affinity of the test substance and the radiolabeled reference estrogen for the ER. Despite the fact that the IC_{50} is very sensitive to experimental conditions while the K_i is less sensitive to these conditions, the majority of investigators present their data as IC_{50} values. This may be due to the fact that the most commonly used approach for comparing data within and between laboratories is the RBA, which is based on IC_{50} values.

The basic procedure proposed by Hähnel (1971) and Korenman (1970), among others, to measure ER binding has been modified over the years by numerous investigators. Common modifications include the source of the ER, the exposure duration and temperature, the reference estrogen, and the adsorbent used to separate the bound, radiolabeled estrogen from unbound molecules. For the purpose of summarizing the available ER binding assay approaches used by different investigators (**Appendix A**), the various protocols have been sorted according to whether they were performed with intact cells, a cell cytosol preparation, or with semi-purified preparations of human or rat ERα or ERβ proteins. The protocols using cytosol have been further categorized according to the source of the cytosol (i.e., rat, mouse, or rabbit uterine

cytosol and cytosol from MCF-7 cells). The data generated from studies using a glutathione construct of the ligand binding domain (def) of the ERα from humans and mouse, and the ER from a lizard (anole), chicken, and rainbow trout were categorized separately.

The first step in an ER binding assay is to determine the K_d of the reference estrogen (e.g., 17β-estradiol) to the ER preparation used in the assay. The purpose of determining the K_d for each ER assay system is to demonstrate that the assay system is valid (e.g., a finite number of high affinity receptors are saturated with ligand) and to optimize the system with respect to receptor and ligand concentration. The K_d is determined in a saturation binding experiment that involves adding increasing concentrations of the radiolabeled reference estrogen to the cells/cytosol and measuring the amount that binds to the ER (Motulsky, 1995). To calculate specific binding of the radiolabeled reference estrogen to the ER, nonspecific binding (i.e., binding to sites other than ER) is measured at each radioligand concentration by the addition of a nonlabeled estrogen at a concentration that occupies all available receptors. The nonspecific binding is subtracted from the total binding (in the absence of nonlabeled compound) of the radiolabeled reference estrogen (Motulsky, 1995). The amount of radioligand specifically bound depends on the number (concentration) of receptors in the preparation. Free and bound radiolabeled ligands are separated by the addition of a nonreactive absorbent, such as dextran charcoal or HAP. The ER, the bound radiolabeled reference estrogen, and other proteins in the reaction mix bind to the absorbent, while the displaced radiolabeled reference estrogen remains in the supernatant. The mixture is centrifuged and the amount of ER-bound radiolabeled reference estrogen in the pellet is measured. The K_d of the reference estrogen, which reflects its affinity for the specific ER preparation, can then be calculated. The K_d is used to determine the appropriate concentration of the reference estrogen to be used in the competitive binding assay.

Because the largest proportion of the published data was derived from studies using uterine cytosol from rats and mice, a general guideline for this method is described first. This guideline is followed with less detailed descriptions of other assays used to measure ER binding. In addition, copies of protocols were requested from investigators using the different *in vitro* ER binding assays; copies of the protocols received for public distribution are provided in **Appendix B**.

2.2 General Overview of *In Vitro* Assays Used To Measure Competitive ER Binding

The primary purpose of most *in vitro* ER binding studies was to investigate the nature of the binding process and the kinetics of the reaction, and to identify which molecular moieties enhanced or inhibited binding to the ER. Thus, most studies were not conducted to specifically identify endocrine disruptors. The publications presenting the results of relevant studies provided various levels of detail on the methods used, ranging from highly specific protocols to a simple listing of the ER source and the identity of the test substances.

A general protocol using uterine cytosol from rats and mice is described below, followed by less detailed descriptions of other assays used to measure ER binding. The majority of the *in vitro* ER binding studies considered for this BRD used 17β-estradiol as the reference estrogen and, thus, this estrogen is included in the general protocols described in the following sections.

2.2.1 Mammalian Uterine Cytosol (Rat, Mouse, Rabbit) as the ER Source

Uterine cytosol is prepared by homogenizing the uterus in cold Tris buffer in a 1:10 ratio of tissue to buffer. The homogenate is centrifuged for 10 minutes at 2,500 x g at 4°C and the pellet containing cell debris is discarded. Next, the supernatant is centrifuged at 105,000 x g for 60 minutes at 4°C to pellet organelles and the cell cytosol supernatant containing the ER is stored at -70°C. Cytosolic protein concentration is determined using conventional methods.

To determine the K_d of 17β-estradiol, radiolabeled (i.e., with ^3H) 17β-estradiol at concentrations ranging from $1.0x10^{-8}$ to $3.3x10^{-11}$ M in buffer is added to aliquots of cytosol. Nonspecific binding of the radiolabeled 17β-estradiol is measured at each concentration by the addition of nonlabeled 17β-estradiol at a concentration that occupies all available receptors. Specific binding to the ER is then calculated at each concentration by subtracting nonspecific 17β-estradiol binding from the total binding of 17β-estradiol. After incubation and separation of bound and unbound radiolabeled 17β-estradiol, the amount of radiolabeled 17β-estradiol bound to the ER is measured. Specific binding data from saturation assays are usually analyzed to obtain the number of binding sites in a cytosolic preparation, B_{max}, and the K_d by nonlinear

regression using log concentration of radiolabeled 17β-estradiol as the independent variable (Motulsky, 1995).

The saturation binding curve of radiolabeled 17β-estradiol can also be analyzed using a linear Scatchard analysis (Scatchard, 1949) with specific binding on the abscissa (usually labeled "Bound") and the ratio of specific binding of 17β-estradiol to free 17β-estradiol (usually labeled "Bound/Free") on the ordinate. In these plots, B_{max} is the x-intercept and K_d is the negative reciprocal of the slope. However, the Scatchard plot is not the most accurate technique to use for analysis because the data is transformed to make a linear graph that is then analyzed by linear regression, and transformation of the data distorts the experimental error. Linear regression analysis assumes that the scatter of points around a line follows a Gaussian distribution so that the standard deviation is the same at every value of X. However, this is not true with transformed data. Secondly, a Scatchard transformation alters the relationship between the "Bound" (X) and the "Bound/Free" (Y) ratio. This is because the value of X is used to calculate the value of Y and this calculation violates the assumptions of linear regression. Thus, the Scatchard values for B_{max} and K_d are often further from their true values than if they had been calculated using nonlinear regression.

To measure competitive binding, radiolabeled 17β-estradiol, at a concentration that approximates the K_d, is added to tubes containing aliquots of the cytosol. A range of concentrations of the test substance in solvent, usually ethanol or unlabeled 17β-estradiol, is added. Nonspecific binding of radiolabeled 17β-estradiol to the receptor is measured by using a 100-fold molar excess of unlabeled 17β-estradiol. Following incubation of the mixture, the displaced radiolabeled 17β-estradiol is separated from the receptor-bound radiolabeled 17β-estradiol using an absorbent, such as dextran charcoal or HAP. The radiolabeled 17β-estradiol-ER complex and the test substance-ER complex bind to the absorbent, and the unbound radiolabeled 17β-estradiol and test substance are removed by extensive washing of the absorbent. After centrifugation, the bound radiolabeled 17β-estradiol in the pellet is extracted with ethanol and the concentration of radiolabel is determined by scintillation counting. Specific binding is calculated by subtracting the amount of nonspecific binding from each sample evaluated in the assay. Data for the binding of the radiolabeled 17β-estradiol and its displacement by each test substance are plotted as the

percentage of radiolabeled 17β-estradiol bound versus the molar concentration of competing test substance. For a substance with high affinity for the receptor, the upper plateau of the curve

$$\text{Specific binding} = \frac{B_{max} \times [\text{Free radiolabeled } 17\beta\text{ - estradiol}]}{K_d + [\text{Free radiolabeled } 17\beta\text{ - estradiol}]}$$

correlates with maximal receptor binding in the absence of the test substance, and the bottom of the curve is the nonspecific binding. The concentration of the test substance that produces radiolabeled 17β-estradiol binding half way between the upper and lower plateaus is the IC_{50}. Estimates of the IC_{50} can be determined using appropriate statistical software.

The K_i, which reflects the affinity of the test substance for the ER, can be calculated from the IC_{50} value using the equation of Cheng and Prusoff (1973):

$$K_i = \frac{IC_{50}}{1 + \frac{[\text{Radiolabeled } 17\beta\text{ - estradiol}]}{K_d}}$$

The RBA value for each competing test substance is calculated by using the following equation:

$$\text{RBA} = \frac{IC_{50} \text{ for } 17\beta\text{ - estradiol}}{IC_{50} \text{ for test substance}} \times 100$$

2.2.2 MCF-7 Cells and MCF-7 Cell-derived Cytosol

2.2.2.1 Intact MCF-7 Cells as ER Source

A number of cell lines inherently contain ER. The cell line most widely used for evaluating ER binding is the human breast adenocarcinoma cell line MCF-7. These cells are maintained in standard growth medium. Prior to their use in ER binding assays, the cells are grown for one to two days in medium containing charcoal-stripped serum. The purpose of charcoal stripping is to remove residual estrogenic substances that may competitively interfere with the binding of reference estrogens and test substances to the receptor.

For testing, intact cells are washed and treated with the radiolabeled 17β-estradiol in serum-free minimal medium. Unlabeled test substances, including 17β-estradiol, are added to the cells under non-growth conditions. Following incubation, the unbound test substance and reference estrogen are removed by washing the cells with ethanol. Scintillation counting is used to determine the extent of binding of the labeled reference estrogen. The amount of radiolabeled reference estrogen displaced by the test substance is used as the measure of its binding affinity for the ER.

2.2.2.2 MCF-7 Cytosol

A cell-free (cytosolic) extract of MCF-7 cells, which is prepared in a similar manner to cytosolic extracts from the rodent uterus, has been used as a source of ER. Cultured MCF-7 cells are harvested, homogenized to disrupt the cell membranes, and centrifuged to separate the nuclear debris and organelles from the cytosol. Generally, the assay is performed as outlined for the uterine cytosol assay.

2.2.3 Semi-Purified ERα and ERβ

In the past few years, researchers have recognized the advantages of using molecular techniques to isolate the ER from mammalian tissues or to clone the DNA coding for the receptor into a plasmid, transfect a cell with the plasmid, and express the ER in a cell. The protein can be isolated and purified, or the cellular extract can be processed such that a semi-purified ER is obtained. Transfected cells or other cell lines with DNA transcripts from different species that code for the complete ER or for selected domains of the ER have been constructed.

Different approaches have been used to produce semi-purified ERα and ERβ proteins for use as receptors to measure binding. To produce these proteins, the cDNA of the corresponding ER genes are cloned into a baculovirus or a transfer vector. The recombinant baculovirus vector is amplified and used to infect insect Sf9 cells (Bolger et al., 1998). Two days after infection, the cells are harvested and nuclei are isolated. A nuclear extract is made with buffer and the concentrations of ER proteins determined based on the specific binding of 17β-estradiol to the receptors in solution (Kuiper et al., 1998). The semi-purified ER preparation compares favorably to the ER isolated from tissue preparations with respect to size, immunogenicity, hormone

binding characteristics, phosphorylation state, and DNA interactions in gel shift assays. This ER also interacts normally with its DNA response element (Cheskis et al., 1997; Ozers et al., 1997).

In some approaches, the cDNA of the ER protein is transcribed, whereas in others, only the cDNA coding for the ligand-binding domain of the ER protein is specifically excised and cloned, and the partial protein is expressed for use in the assay. Another approach has been the use of a rabbit cell expression lysate to produce the ERα and ERβ proteins. The semi-purified ER proteins are produced by cloning the receptor genes into a plasmid, followed by the synthesis of the protein using the TnT-coupled reticulocyte lysate system with T7-RNA polymerase. Aliquots of the translation reaction mixture are used in the competitive binding assay (Kuiper et al., 1997).

2.2.3.1 Solid Phase Ligand Binding Assay using ScintiStrip™ (Kuiper at al., 1998)

The wells of ScintiStrip™ microtiter plates have scintillation fluors incorporated into the plastic. Signal detection is based on the premise that tritium (^{3}H) is a weak β emitter and low energy electrons have a short range in solution; the tritiated molecules, binding to the solid support containing the fluor, will trigger a response. The assay is performed by binding the ERα and ERβ proteins to the plastic support, followed by the addition of radiolabeled 17β-estradiol and the test substance. Scintillation counting of the wells will detect only the radiolabeled 17β-estradiol that remains bound to the ER, whereas radiolabeled 17β-estradiol displaced from the ER by the test substance will not be detected.

2.2.4 GST-ERdef Fusion Proteins

GST-ERdef fusion proteins contain only the ligand binding domain (known as the def domains) of the ER fused to glutathione-*S*-transferase (GST). The def domains have been transcribed and translated for use in measuring the ER-binding of 17β-estradiol and other substances. This approach has been used to prepare the partially purified binding domains of the ER protein from the lizard (anole), chicken, and rainbow trout, and the ERα from human and mouse (Matthews et al., 2000; Matthews and Zacharewski, 2000).

Essentially, assays using GST-ERdef proteins are performed as described above for cytosol except incubations are in 1 mL glass tubes arranged in a 96-well format. Bound radiolabeled 17β-estradiol is separated from free radiolabeled 17β-estradiol using a 96-well filter plate and vacuum pump harvester. The filter plates containing the protein are washed with buffer and the plates are allowed to dry under continuous suction. After drying, the undersides of the filter plates are sealed and scintillation cocktail is added to each well. Bound radiolabeled 17β-estradiol is measured using a scintillation counter. Nonspecific binding of 17β-estradiol is determined in the presence of a 400-fold excess of unlabeled 17β-estradiol (Matthews and Zacharewski, 2000).

2.2.5 Fluorescent Polarization (FP)

2.2.5.1 Theory of FP

FP is a technique that can detect molecular interactions by monitoring changes in the size of fluorescently labeled or inherently fluorescent molecules (Dandliker et al., 1981; Checovich et al., 1995; Jameson and Sawyer, 1995; Lundblad et al., 1996). When a fluorescent molecule binds to another molecule, its speed of rotation changes. This change in speed or tumbling rate can be quantified by FP. When a solution of fluorescent molecules is excited by plane-polarized light, those molecules parallel to the plane become excited. If the molecules remain stationary during the period of excitation (4 nanoseconds for fluorescein), the emitted light remains highly polarized. However, if the molecules tumble during the period of excitation, the emitted light will be random or depolarized. An increase in the volume or conformation of a fluorescent molecule (e.g., through its binding to a receptor or antibody) or a decrease in its conformation or molecular volume (due to dissociation or enzymatic degradation) can be directly measured by FP. The observed value is a weighted average of the polarization values of the individual bound and free fluorescent molecules, and is therefore a direct measure of the fraction bound. The concentration of the bound ligand is derived from the polarization value, and the resultant bound versus free isotherm is analyzed in a similar manner to the graph generated by conventional techniques for radioactivity (Dandliker et al., 1981; Checovich et al., 1995; Jameson and Sawyer, 1995).

For the FP assay, purified, full-length hERα or hERβ and an intrinsically fluorescent nonsteroidal estrogen (Fluormone ES1; FES1), which binds to the hER with high affinity, are used (Bolger et al., 1998). This particular fluorescent estrogen was developed by Katzenellenbogen and colleagues (Hwang et al., 1992).

In the competitive binding assay, substances are tested for their ability to displace the fluorescent ligand FES1 from an ER-FES1 complex. The large ER-FES1 complex tumbles slowly and therefore has a high anisotropy value. As increasing concentrations of a competing ligand displace the FES1 from the complex, the free FES1 molecules tumble more rapidly and have a lower anisotropy value. As more FES1 molecules are displaced from the complex, the measured anisotropy approaches the free anisotropy value. The measured anisotropy is a weighted average of the bound and free FES1 molecules.

2.2.5.2 Conduct of the Assay

As described for the other assays, a binding constant of reference estrogen, in this case FES1, to the receptor must be determined. This approach ensures that a saturating concentration of FES1 is used in the competitive binding assay. The receptor is serially diluted and the same concentration of FES1 is added to each tube. After incubation at room temperature, the fluorescence anisotropy of each tube is measured with a 360 nm excitation filter and a 530 nm emission filter. The anisotropy at each ER concentration is converted to the fraction of ligand bound using the following equation:

$$F_b = \frac{A - A_f}{A_b - A_f}$$

where F_b is the fraction of ligand bound, A is observed anisotropy, and A_b and A_f are anisotropy values of the bound and free ligand, respectively.

Bound ER (ER_b) is assumed to be equal to bound ligand (L_b), and therefore determined by multiplying F_b by the total ligand concentration (L_t). Free ER (ER_f) is calculated by subtracting ER_b from the total ER in the assay (ER_t). The equilibrium binding constant, K_d, is calculated from the ER-bound versus ER-free isotherm using a nonlinear least-square curve fitting program.

In performing the competitive binding assay, aliquots of the serially diluted test compound are added to known concentrations of hERα and FES1. Negative controls containing hERα + FES1 (equivalent to 0% inhibition), and positive controls containing free FES1 (equivalent to 100% inhibition), in the absence of competitor, are included in each run. Varying concentrations of the competitive ligand are added to tubes containing the same concentrations of hERα and FES1. After incubation at room temperature, the anisotropy value in each tube is measured. The anisotropy values are converted to percent inhibition using the following formula:

$$I\% = \frac{A_o - A}{A_o - A_{100}} \times 100$$

where A_o, A_{100}, and A are the percent inhibition, A_o at 0% inhibition, A_{100} at 100% inhibition, and the observed A value, respectively.

Polarization values are converted to percent inhibition to normalize day-to-day differences in the starting 0% inhibition polarization values. The percent inhibition versus competitor concentration curves is analyzed by nonlinear least-squares curve fitting to yield an IC_{50} value. IC_{50} values are converted to an RBA value using 17β-estradiol as a standard; the RBA value using 17β-estradiol is set to 100.

2.2.6 Permutations of the Assays as Described in the Literature

Irrespective of source of the ER used in a particular study, the protocols vary from laboratory to laboratory. Some of these variations are in response to the differing properties of the ER preparations used, or because of various questions the studies were designed to address. The permutations in the protocols used by each laboratory for each source of ER are summarized in **Appendix A**.

3.0 CHARACTERIZATION OF SUBSTANCES TESTED IN ER BINDING ASSAYS

3.1 Introduction

ER binding data were obtained for a total of 638 substances **(Appendix C)**. While a relatively large number of substances have been tested in ER binding assays, only a small number of these substances were evaluated in multiple types of ER binding assays and/or by multiple laboratories. With the exception of 17β-estradiol, the reference estrogen used in most studies, only 14 substances (2.2%) were tested in at least 10 of the 14 assays considered in this BRD. These substances are bisphenol A, 2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane (HPTE), kepone, coumestrol, *o,p'*-dichlorodiphenyltrichloroethane (*o,p'*-DDT), diethylstilbestrol (DES), 5α-dihydrotestosterone, estriol, estrone, 4-hydroxytamoxifen, genistein, methoxychlor, tamoxifen, and zearalenone. Ninety-four percent (600) of the substances in the database were tested in five or fewer assays, 63% (403) were tested in one assay only, and 59% (376) were tested in one publication only.

3.2 Rationale for Selection of Substances/Products Tested in *In Vitro* ER Binding Assays

Most of the substances tested in *in vitro* ER binding assays closely parallel the initial studies on the isolation and characterization of the receptor, the subsequent synthesis and characterization of ER agonists and antagonists, and the more recent use of ER binding assays as a method for endocrine disruptor screening. Many of the first substances to be tested were selected to address basic research questions regarding the nature of the ER and the kinetics of its interactions. A number of the triphenylethylenes, stilbenes, and DES analogs and derivatives, for example, were investigated to obtain a better understanding of ER binding processes. Some substances were investigated in research and development studies designed to determine which metabolite or stereoisomer of a molecule enhanced or inhibited binding to the ER. Data from these types of studies often contributed to the development of pharmaceuticals for breast cancer, estrogen-replacement therapy, or for other health concerns. Some substances were investigated to determine structure-activity relationships (SAR) for the development of quantitative SAR (QSAR) models. Finally, during the last decade, with the growing concern about possible adverse health effects associated with exposure to endocrine disruptors, some of these substances (e.g., pesticides, polychlorinated biphenyls, phytoestrogens) were tested using *in vitro* ER

binding assays to identify those that may act as estrogen agonists/antagonists in humans and wildlife.

3.3 Chemical and Product Classes Tested

Chemical and product class information for the substances tested in ER binding assays is provided in **Appendix C**. Substances were assigned to a single chemical class based on available information from standardized references (e.g., *The Merck Index 12th Edition* and the U.S. National Library of Medicine's ChemID database) and from an assessment of chemical structure. As shown in **Table 3-1**, the chemical classes with the greatest amount of *in vitro* ER binding data are polychlorinated biphenyls, phenolic and nonphenolic steroids, triphenylethylenes, organochlorines, polycyclic aromatic hydrocarbons, stilbenes, phenols, and bisphenols. Of the 638 substances included in **Appendix C**, seven substances were not classified within a chemical class.

Product classes were assigned based on information contained in *The Merck Index* and the U.S. National Library of Medicine's ChemFinder. As show in **Table 3-2**, the most common product classes tested in *in vitro* ER binding assays have been pharmaceuticals, pesticides, chemical intermediates, dielectric fluids or their components, natural products (including several phytoestrogens), and plasticizers. Of the 638 substances included in **Appendix C**, 320 were not classified within a product class.

Table 3-1 Chemical Classes Tested in *In Vitro* ER Binding Assays (638 Substances)

Chemical Class	# of Substances
Acetamide	2
Acrylate	6
Alcohol	4
Aldehyde	1
Alkoxyphenol	5
Alkylbenzene	2
Alkylphenol	14
Amide	1
Anilide	2
Aniline	4
Aromatic amine	1
Aromatic heterocycle	1
Aromatic hydrocarbon	1
Azo compound	1
Benzophenone	6
Biphenyl	3
Biphenyldiol	1
Bisphenol	27
Carbamate	3
Carboxylic acid	4
Chalconoid	6
Chlorinated aromatic hydrocarbon	1
Chlorinated bridged cycloalkene	2
Chlorinated cycloalkane	1
Chlorinated cyclodiene	4
Chlorinated phenol	4
Coumarin	1
Crown ether	1
Cyclodiene	1
Dioxin	1
Diphenolalkane	18
Diphenolalkene	4
Diphenyl ether	2
Diphenylalkane	5
Ester	1
Flavanone	10
Flavone	14
Glucuronide	1
Heterocyclic aromatic aldehyde	1
Imidazole	1
Indane	1
Indene	15
Isoflavone	15
Nitrobenzene	1
Nitrogen heterocycle	2
Organochlorine	44
Paraben	7
Phenol	40
Phenoxy carboxylic acid	1
Phosphate ester	1
Phthalate	13
Phthalimide	1
Piperidine	1
Polychlorinated biphenyl (PCB)	93
Polycyclic aromatic hydrocarbon	42
Polyether	1
Pteridine	1
Purine	1
Pyrazole	1
Pyrethrin	6
Pyrethroid	6
Resorcylic acid lactone	6
Siloxane	2
Steroid, nonphenolic	58
Steroid, phenolic	69
Stilbene	40
Sulfoxide	1
Terpene	2
Tetrahydrophenanthrene	1
Thiophene	2
Triazine	9
Triphenylethylene	49
Triphenylmethane	2
Not classified	7

Table 3-2 Product Classes Tested in *In Vitro* ER Binding Assays (638 Substances)

Product Class	Number of Substances
Analytical reagent	1
Antioxidant	1
Chemical additive	2
Chemical intermediate (adhesive, coatings, cosmetic, dye, elastomer, fiber, film, flavor, fragrance, lubricant, monomer, pesticide, plasticizer, pharmaceutical, polyester, polymer, resin, surfactant)	57
Dielectric fluid or component	54
Dye	4
Flavor	2
Food additive	16
Fragrance	6
Lubricant additive	1
Natural product (plant or animal)	31
Pesticide/Pesticide metabolite	61
Pharmaceutical/Pharmaceutical metabolite/ Pharmaceutical additive	92
Plant growth regulator	1
Plasticizer	16
Polymer	2
Preservative	5
Solvent	4
Surfactant	1
Not classified	320

4.0 REFERENCE DATA

The ability of a test substance to bind to the ER *in vitro*, whether to an isolated protein receptor molecule or to ERs in cultured cells, suggests, but does not demonstrate, the ability of the substance to act as an estrogen agonist or antagonist. A commonly used *in vitro* method to measure such biological effects is based on an assessment of the ability of a substance to induce or inhibit transcriptional activation of an ER-dependent reporter gene function.

The purpose of this BRD is to assess the sensitivity of various *in vitro* ER binding assays for detecting ER-binding substances with various binding activities and to assess reliability within and among laboratories and across procedures. No attempt is made to evaluate their performance with respect to other *in vitro* biological end points, such as transcriptional activation, or *in vivo*, such as promotion of uterine growth. Such comparisons will be addressed elsewhere. Therefore, no reference data are included for assessing the biological relevance of the ER binding assays.

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5.0 DATA ON *IN VITRO* ER BINDING ASSAYS

5.1 Introduction

Methods and ER binding data were collected from 72 publications reporting studies in which the competitive binding of a substance to the ER was measured and RBA values were included or could be calculated. When provided, the specific information extracted for each substance included its name, source, purity, methodological details, relevant binding data (K_i, IC_{50}, and/or RBA values for positive studies, highest dose tested [HDT] for negative studies), and the citation. For studies in which chemical structures only were provided, every effort was made to identify the name of each substance tested. No attempt was made to identify the source and purity of a substance if the investigators did not provide such information. If available, a Chemical Abstract Service Registry Number (CASRN) was entered for each substance. This identifier was obtained from various sources, including the publication, the National Library of Medicine's ChemID database, and *The Merck Index*. Chemical name synonyms were entered for substances that were identified in the literature by more than one name, and for substances where the literature name may have been different from the generic name. All substances with the same CASRN were listed under the same name, regardless of the name that was used in the original publication. **Appendix C** provides information on the names, synonyms, CASRN, and chemical/product class, where available, for each substance, while **Appendix D** contains the *in vitro* ER binding data sorted alphabetically by substance name.

5.2 Availability of Detailed *In Vitro* ER Binding Protocols

The scientific methods presented in the publications containing data from competitive *in vitro* ER binding studies provided various levels of detail. To the extent possible, the most important method parameters were extracted from each publication and summarized in **Appendix A**. Details about the following method parameters are included in the Appendix to the extent this information was available:

- Preparation of the receptor (e.g., species or cell line, buffer used for preparation of cytosol, protein concentration of cytosol);
- Competitive binding assay (e.g., concentration of radiolabeled estrogen, solvent used to dissolve the test substance, concentration range of the test substance, number of replicates within an assay, number of times assay was repeated);

- Separation of ligand (e.g., type of slurry used, incubation time, temperature); and
- Data calculations (e.g., method used for calculating data, data format).

5.3 Availability of *In Vitro* ER Binding Data

ER binding data were collected for a total of 638 substances tested in competitive binding studies with ER obtained from the following sources:

1. Rat uterine cytosol (RUC);
2. Mouse uterine cytosol (MUC);
3. Rabbit uterine cytosol (RBC);
4. Cytosol from human adenocarcinoma MCF-7 cells (MCF-7 cytosol);
5. Intact MCF-7 cells (MCF-7 cells);
6. Semi-purified human ERα protein (hERα);
7. Semi-purified human ERβ protein (hERβ);
8. Semi-purified rat ERβ protein (rERβ);
9. Semi-purified human ERα as measured by FP (hERα-FP);
10. Glutathione-*S*-transferase fusion proteins consisting of the def domains of the human ERα receptor (GST-hERαdef);
11. Glutathione-*S*-transferase fusion proteins consisting of the def domains of the mouse ERα receptor (GST-mERαdef);
12. Glutathione-*S*-transferase fusion proteins consisting of the def domains of the lizard (anole) (GST-aERdef);
13. Glutathione-*S*-transferase fusion proteins consisting of the def domains of the chicken (GST-cERdef); and
14. Glutathione-*S*-transferase fusion proteins consisting of the def domains of the rainbow trout (GST-rtERdef).

In all studies, competitive binding was measured by the displacement of radiolabeled ([^{3}H] or [^{131}I]) 17β-estradiol from the ER-estrogen complex or by the change in anisotropy of the fluorescent ER-estrogen complex by the test substance. **Appendix D** presents the extracted and compiled data sorted first by substance name and then by assay. In those cases in which the RBA value was not provided in the citation, this value was calculated, when possible, from

provided IC_{50} values. Not all of these values were reported in all publications. In some publications, neither the IC_{50} nor the RBA values were presented. In many of these cases, the binding of the test substance to the ER over a range of concentrations was presented graphically, so that the IC_{50} values of 17β-estradiol and the test substance could be estimated. These estimated IC_{50} values and corresponding calculated RBA values are italicized in **Appendix D**. For substances that did not bind sufficiently well to the ER to displace the reference estrogen (i.e., an IC_{50} value could not be calculated), the only parameter that could be entered into the database was the HDT.

5.4 *In Vitro* ER Binding Assay Results for Individual Substances

The number of *in vitro* ER binding assays in which each substance was tested is provided in **Appendix E**. These data, shown in **Table 5-1,** are summarized by assay and ranked according to the number of substances tested. Of the 638 substances tested in the 14 different *in vitro* ER binding assays, the majority of substances (376 or 59%) had been tested in the RUC assay. Only 133 (21%) of these substances had been tested in the next most frequently used assay, hERα. For five of the 14 assays (hERα-FP, RBC, rERβ, GST-mERαdef, GST-cERdef), published data on less than 50 substances for each assay were located.

As presented in **Table 5-2**, only 14 (excluding the reference compound 17β-estradiol) of the 638 substances (2.4%) had been tested in 10 or more assays, and of these, only three substances (0.47%) had been tested in all 14 assays. As stated in **Section 3**, 94% (600) of the substances in the database had been tested in one to five assays, with 63% (403) tested in one assay only.

5.5 Use of Coded Chemicals and Compliance with Good Laboratory Practice (GLP) Guidelines

Based on the available information in the scientific literature, it appears that the published *in vitro* ER binding assay studies neither used coded chemicals nor were they conducted in compliance with GLP guidelines (see **Section 8**).

Table 5-1 Number of Substances Tested in Various *In Vitro* ER Binding Assays (638 Substances)*

Assay	Number of Substances Tested	% of Total Substances Tested
RUC	376	59%
hERα	133	21%
hERβ	101	16%
GST-hERαdef	99	16%
MCF-7 cytosol	94	15%
GST-rtERdef	86	13%
GST-aERdef	85	13%
MUC	75	12%
MCF-7 cells	66	10%
hERα-FP	48	8%
RBC	45	7%
rERβ	37	6%
GST-mERαdef	34	5%
GST-cERdef	34	5%

*Assays sorted according to the number of substances tested.

Table 5-2 Substances Tested in Ten or More *In Vitro* ER Binding Assays*

Substance	Number of Assays	Number of Publications
17β-Estradiol	14	72
Diethylstilbestrol	14	30
Bisphenol A	14	22
Tamoxifen	14	13
Estrone	13	12
p,p'-Methoxychlor	13	11
4-Hydroxytamoxifen	13	10
o,p'-DDT	12	9
Estriol	12	9
Genistein	11	9
Coumestrol	11	7
Kepone	10	7
2,2-Bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane (HPTE)	10	7
5α-Dihydrotestosterone	10	6
Zearalenone	10	5

*Substances sorted by the number of assays tested and then by the number of publications.

6.0 *IN VITRO* ER BINDING TEST METHOD PERFORMANCE ASSESSMENT

6.1 Introduction

The ICCVAM Submission Guidelines (ICCVAM, 1999) request that an assessment be conducted of the performance (i.e., accuracy, sensitivity, specificity, positive and negative predictivity, and false positive and false negative rates[1]) of the proposed test method with respect to its ability to predict the effect of interest in the reference test method currently accepted by the regulatory agencies and, where feasible, to predict adverse health outcomes in the species of interest (e.g., humans, wildlife). Currently, there are no validated *in vivo* reference test methods developed to specifically assess the ability of a test substance to disrupt endocrine function, and data on endocrine disruption in humans or wildlife are too limited to be used for this purpose. Therefore, the existing *in vitro* ER binding assays were compared against each other with regard to their ability to detect substances capable of binding to the ER. However, this type of analysis of *in vitro* ER binding assays is limited by the lack of multiple test data within and across assays for most of the substances considered, and by the paucity of data for the same substances tested in multiple assays.

Taking these limitations into account, a comparative evaluation was conducted of the relative performance of the 14 *in vitro* ER binding assays considered in this BRD. Both quantitative and qualitative assessments of IC_{50} and RBA values were conducted. The quantitative assessment was based on the 238 substances (37.3% of the 638 substances in the *in vitro* ER binding assay database) that had been tested in at least two assays (**Appendix E**), and was further limited to individual tests that resulted in an IC_{50} or RBA value (i.e., the substance was classified as positive). The qualitative assessment was limited to the 100 substances that had been tested in the RUC assay and in at least one of the 13 other *in vitro* ER binding assays, and included substances classified as negative for ER-binding activity.

[1] Accuracy is defined as the proportion of correct outcomes of a method, often used interchangeably with concordance; Sensitivity is defined as the proportion of all positive substances that are correctly classified as positive in a test; Specificity is defined as the proportion of all negative substances that are correctly classified as negative in a test; Positive predictivity is defined as the proportion of correct positive responses among substances testing positive; Negative predictivity is defined as the proportion of correct negative responses among substances testing negative; False positive rate is defined as the proportion of all negative substances that are falsely identified as positive; False negative rate is defined as the proportion of all positive substances that are falsely identified as negative (NIEHS, 1997).

Table 6-1 Number of Substances Tested in Multiple *In Vitro* ER Binding Assays

Number of Assays	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Number of Substances	403	87	74	23	13	6	7	5	5	4	2	2	3	4	638
% of Substances	63.2	13.6	11.6	3.6	2..0	0.9	1.1	0.8	0.8	0.6	0.3	0.3	0.5	0.6	100

6.2 Quantitative Assessments of Assay Performance

To reduce the extent of skewness in the data prior to conducting the quantitative assessments, the two outcome variables for *in vitro* ER binding assays — the RBA and the IC_{50} values — were transformed using the natural log. Studies that did not result in an IC_{50} and/or RBA value were eliminated from consideration. Given the large number of data points for modeling, the general linear models (GLM) used in this analysis are robust, although some skewness may yet exist with the data. To simplify the comparison, each literature citation was considered an independent assessment (designated here as a 'reference').

Two-way and three-way analysis of variance models were performed with random effects to estimate the intra-class correlation of substances. A high correlation value indicates that the lnRBA or $lnIC_{50}$ values are more similar within groups than among groups, where groups can be defined by assay or by reference. Estimates of variance for each model component and intra-class correlation are presented to show which factors (substance, assay, or reference) are responsible for the greatest variation in the lnRBA and $lnIC_{50}$ values. Due to limitations in the database with regard to the number of substances tested in multiple assays and to the number of independent tests performed for the substance using the same assay, the results of these analyses must be viewed with caution.

Initially, all data representing all substances, assays, and references were considered, and unique data (i.e., substances tested only in a single assay) were excluded from subsequent analyses. For the analysis of lnRBA values, a total of 752 data points representing 211 substances, 14 assays, and 51 references were considered. For the analysis of $lnIC_{50}$ values, 369 data points representing 119 substances, 13 assays, and 31 references were considered. The $lnIC_{50}$ and the lnRBA values for 17β-estradiol were omitted from these analyses. The RBA values for 17β-

estradiol are uninformative because they are arbitrarily set at 100% in all assays in which this substance is used as the reference estrogen. The IC_{50} values for 17β-estradiol represent the largest collection of IC_{50} data for a single substance and were evaluated independently to avoid potentially biasing the quantitative analysis.

6.2.1 Measures of Intra-Class Correlation

The intra-class correlation, r_I, measures the percentage of variation in y, the outcome variable, explained by a given component or set of components. The model is y = substance + assay + reference. **Table 6-2** contains the components of variance for each variable adjusted for the other two variables. Interpretation of this analysis is limited to factors that impact on performance; factors that impact on assay reliability are discussed in **Section 7**.

From this analysis, it appears that the lnRBA or $lnIC_{50}$ values for a specific substance were generally consistent irrespective of which assay was used or which laboratory conducted the study. The greatest variation in lnRBA or $lnIC_{50}$ values was found between substances (i.e., the most important parameter was the intrinsic ER binding property of the substance). The greater contribution of substances to the overall variance is not surprising considering the seven orders of magnitude range in reported $IC_{50,}$ and thus RBA, values.

6.2.2 Evaluation of Substances Tested in Nine or More *In Vitro* ER Binding Assays

In this analysis, the variances for the RBA values of the 12 substances that had been tested in at least nine of the 14 *in vitro* ER binding assays were determined. Although 14 substances (excluding 17β-estradiol) had been tested in at least ten *in vitro* ER binding assays (**Section 5**), only those substances that elicited a positive response in at least one experiment in each assay could be used in this analysis. The variances and sample sizes for these 12 substances are provided in **Table 6-3,** ranked in descending order according to the median RBA value based on all test data. Only assays for which variances could be calculated are included, and most of these variances were based on three or four values only. Due to the lack of sufficient data, a corresponding analysis of IC_{50} values was not conducted.

Table 6-2 Components of Variance for Each Variable Adjusted for the Other Two Variables – Performance Assessment

	Outcome, y (% variation)	
	lnRBA	$lnIC_{50}$
Var(substance)	8.34	8.49
Var(assay)	0.38	0.34
Var(reference)	1.40	2.01
Var(error)	1.75	2.44
Corr (y_{ijk}, $y_{ij'k'}$)*	0.70	0.64
Corr (y_{ijk}, $y_{ijk'}$)**	0.73	0.67
Corr (y_{ijk}, $y_{ij'k}$)***	0.82	0.79

*A high correlation was found for the lnRBA values within substances using any assay or reference (i.e., the lnRBA values are more correlated within than across substances). A slightly lower correlation was found when $lnIC_{50}$ values were used. The high correlation for the lnRBA values suggests that the RBA of a specific substance to the ER did not vary much among the different binding assays.
**This correlation suggests that the test substances responded similarly in an assay irrespective of the laboratory in which the test was conducted. Variation within laboratories is slightly less than the variation across laboratories.
*** A high correlation was found for substances tested in the same laboratories (i.e., references) but using different assays.

A large p value (p1 or p2) identifies those substances, such as zearalenone, estriol, estrone, diethylstilbestrol (DES), 2,2-bis(*p*-hydroxyphenyl)-1,1,1,-trichloroethane (HPTE), bisphenol A, and kepone, with the least amount of variability in their lnRBA values. In contrast, the p1 values of coumestrol and tamoxifen are below 0.05, indicating that significant variability exists across assays irrespective of the laboratory in which the tests were performed. A possible explanation for the variability with coumestrol, a phytoestrogen, is its ~1.5-log greater binding affinity to the ERβ protein compared to the ERα protein (**Appendix D**). No explanation can be provided for the significant variability in lnRBA values for tamoxifen. Values for p2 could not be calculated in every case since there were too few assays or references that could be used in the analysis. A significant p2 value was not found for any substance suggesting that there was not significant variability due to the reference (i.e. laboratories in which the substance was tested).

Another approach to evaluating the variability across assays for a substance is to fit a two-way model, where y = assay + reference. In this analysis (**Table 6-4**), adjustment is made for inter-reference variation in lnRBA so that only those assays used twice or more in two or more

Table 6-3 Variance of lnRBA Values by Substance and Assay – Performance Assessment[a]

Substance[b] (CASRN)	Median[c] RBA	#of Obs/ # Assays	hERα[d]	hERα-FP[d]	hERβ[d]	MCF-7 cytosol[d]	MUC[d]	RUC[d]	p1*	p2**
4-Hydroxy-tamoxifen (68047-06-3)	168	18/13	0.28 (3)	1.82 (3)					0.08	0.15
DES (56-53-1)	127	38/14	0.99 (3)	0.45 (4)			0.60 (7)	3.42 (11)	0.15	0.99
Estrone (53-16-7)	45	18/13				2.40 (3)		0.98 (4)	0.73	na[e]
Estriol (50-27-1)	15.8	16/12				2.42 (4)			0.53	0.64
Zearalenone (17924-92-4)	15.0	11/9	All n≤2						0.42	na
Tamoxifen (10540-29-1)	5.0	21/14	0.44 (3)					2.01 (4)	0.02	0.10
Coumestrol (479-13-0)	3.1	15/11	0.79 (3)						0.02	0.25
HPTE (2971-36-0)	1.45	12/10						1.53 (3)	0.82	na
Genistein (446-72-0)	1.30	18/11	1.07 (4)		0.97 (3)				0.11	0.18
Bisphenol A (80-05-7)	0.031	22/14	1.36 (3)					1.25 (5)	0.53	0.60
o,p'-DDT (789-02-6)	0.038	15/10						1.72 (5)	0.20	na
Kepone (143-50-0)	0.027	11/9						1.39 (3)	0.60	na

[a]Only assays where a variance could be calculated for at least one of the 12 substances are listed. The variance for a particular assay could be calculated only if a particular substance was tested three or more times in that assay; empty cells indicate insufficient data to calculate a variance. The p values could be calculated only if there were two observations from at least three or more assays; a missing p-value indicates insufficient data.

[b]Substances that had been tested in at least 9 of the 14 *in vitro* ER binding assays; DES = diethylstilbestrol; *o,p'*-DDT = *o,p'*-dichlorodiphenyltrichloroethane; HPTE = 2,2-Bis(*p*-hydroxyphenyl)-1,1,1,-trichloroethane.

[c]The median RBA value across assays, based on positive test data.

[d]The numbers in parenthesis indicate the numbers of replicate tests.

[e] na = No p value could be calculated since there was either no values or only one value per assay x response combination.

*p1 tests whether there is a significant difference among all assays used; unadjusted for references.

**p2 tests whether there is a significant difference among all assays used; adjusted for references.

laboratories (references) are considered. Results are presented in descending order according to the median RBA value across assays, based on all positive test data, for each of the 12 substances. The components of the variance for each variable are adjusted for the other variable. Due to the lack of sufficient data, a corresponding analysis of IC_{50} values was not conducted.

Table 6-4 Variance for Y=lnRBA Values

Substance[a] (CASRN)	Median RBA[b]	N[c]	n/n'[c]	var(assay)	var(ref)	var(error)	r_I[d](assay)
4-Hydroxy-tamoxifen (68047-06-3)	168	18	13/8	0.66	1.58	0.17	0.27
DES (56-53-1)	127	38	14/8	≤0.001	6.37	0.35	~0[e]
Estrone (53-16-7)	45	18	13/7	0.25	2.88	0	0.08
Estriol (50-27-1)	15.8	16	12/7	0.096	4.54	0.49	0.001
Zearalenone (17924-92-4)	15.0	11	9/6	0.27	Too few references	0.44	0.38
Tamoxifen (10540-29-1)	5.0	21	14/8	0.53	1.91	0.08	0.21
Coumestrol (479-13-0)	3.1	15	11/7	0.49	0.22	0.43	0.43
HPTE (2971-36-0)	1.45	12	10/6	1.14	2.34	0	0.33
Genistein (446-72-0)	1.30	18	11/7	1.41	≤0.001	1.23	0.53
o,p'-DDT (789-02-6)	0.038	15	10/4	2.89	2.90	0	0.50
Bisphenol A (80-05-7)	0.031	22	14/8	≤0.001	≤0.001	2.64	~0
Kepone (143-50-0)	0.027	11	9/6	0.84	1.93	0	0.30

[a]Substances that had been tested in at least nine of the 14 *in vitro* ER binding assays; DES = diethylstilbestrol; *o,p'*-DDT = *o,p'*-dichlorodiphenyltrichloroethane; HPTE = 2,2-Bis(*p*-hydroxyphenyl)-1,1,1,-trichloroethane

[b]The median RBA value across assays, based on positive test data.

[c]N is the total number of values available; n is the number of assays used to test that substance; and n' is the number of assays that can be adjusted for the effect of reference to generate the data in this table.

[d]r_I, the intra-class correlation, measures the percentage of variation in y, the outcome variable, explained by a given component or set of components

[e]$r_{I=}$ 0 when each RBA value is derived from a different assay x reference combination

As demonstrated by the relatively small intra-class correlation values, the lnRBA values are very similar across assays for estriol and estrone, and not quite as similar across assays for tamoxifen, HPTE, kepone, and 4-hydroxytamoxifen. The relatively large intra-class correlation values for genistein, coumestrol, *o,p'*-DDT and zearalenone suggest that these substances respond differently in the various assays. The explanation for the increased variability associated with genistein and coumestrol, both of which are phytoestrogens, might be their ~1.5-log greater binding affinity to the ERβ protein compared to the ERα protein used in other assays. No explanation can be provided for the increased variability in lnRBA values associated with zearalenone and *o,p*'-DDT. However, the lack of an obvious relationship between the magnitude of the median RBA value for a substance and its intra-class correlation value suggests that the increased variability across assays for some substances is not a reflection of its binding activity. This analysis is affected to a great extent by the fact that so few assays were used within the same reference.

6.2.3 Variability in $lnIC_{50}$ and lnRBA Values for Selected Substances

Another approach for assessing the variability between substances is to evaluate the standard deviation of the lnRBA and $lnIC_{50}$ values of the 12 substances tested in at least nine of the 14 *in vitro* ER binding assays. These data are tabulated along with the corresponding median RBA values across assays in **Table 6-5**. The standard deviations were visually compared to determine which substances demonstrate more variability than others if the effects of assay and laboratory, which appear to be relatively small, are ignored. The overall variability presented in **Table 6-5** and the variability across and within assays shown in **Table 6-4** should be considered together.

The least amount of variation in binding affinity (based on assessing both lnRBA and $lnIC_{50}$ values) occurred for zearalenone, while the greatest variations (twice the lowest value) were observed for coumestrol, *o,p*'-DDT, and DES. Among the other substances, the variability in binding affinity was relatively similar among the different assays. Increased variability in the lnRBA and $lnIC_{50}$ values for coumestrol may be related to its much higher binding affinity for the purified proteins, especially ERβ, compared to the cytosolic receptors (**Appendix D**).

Table 6-5 Variability in Standard Deviations for lnRBA and $lnIC_{50}$ Values For Selected Substances

Substance[a] (CASRN)	Median[b] RBA	# of Assays	lnRBA		$lnIC_{50}$	
			Standard Deviation	N[c]	Standard Deviation	N[c]
4-Hydroxy-tamoxifen (68047-06-3)	168	13	1.36	18	1.68	10
DES (56-53-1)	127	14	2.01	38	3.20	26
Estrone (53-16-7)	45	13	1.49	18	1.57	8
Estriol (50-27-1)	15.8	12	1.36	16	0.89	6
Zearalenone (17924-92-4)	15.0	9	0.84	11	0.76	8
Tamoxifen (10540-29-1)	5.0	14	1.91	21	1.68	13
Coumestrol (479-13-0)	3.1	11	2.30	15	2.51	9
HPTE (2971-36-0)	1.45	10	1.15	12	1.14	10
Genistein (446-72-0)	1.30	11	1.74	18	1.64	12
o,p'-DDT (789-02-6)	0.038	10	2.27	15	1.87,	12
Bisphenol A (80-05-7)	0.031	14	1.63	22	1.54	15
Kepone (143-50-0)	0.027	9	1.37	11	1.07	8

[a]Substances that had been tested in at least 9 of the 14 *in vitro* ER binding assays; DES =diethylstilbestrol; *o,p'*-DDT=*o,p'*-dichlorodiphenyltrichloroethane; HPTE=(2,2-Bis(*p*-hydroxyphenyl)-1,1,1,-trichloroethane.
[b]The median RBA value across assays, based on positive test data.
[c]N indicates the number of RBA or IC_{50} values used in the analysis.

6.3 Qualitative Assessment of *In Vitro* ER Binding Assay Performance

A qualitative comparative assessment of assay performance considered the relative ability of the 14 *in vitro* ER binding assays to identify substances with relatively weak ER binding affinities and to obtain higher RBA values for the same set of substances. In conducting this assessment, it was assumed that all positive study results and all negative results for studies in which the highest dose tested was at least 100 μM were correct, for that assay. The 100 μM dose level criterion for negative studies was used to ensure that the protocol (in terms of test substance dose

levels) was minimally adequate for detecting weak positive responses. Thus, a positive assay reflects the intrinsic ability of the test substance to bind to the ER while a negative assay reflects difference in assay sensitivity rather than differences in the experimental protocol.

Due to the RUC assay having the largest database, this assay was used as the standard to compare with the performance of each of the 13 other *in vitro* ER binding assays. To conduct this assessment, the median RBA value was calculated for any substance tested positive in two or more tests using the same assay; otherwise the RBA value for a single positive test was used for that assay. Next, the resulting single or median RBA value for each substance in each assay was classified into one of seven RBA activity categories -- ≥100, from <100 to 10, from <10 to 1, from <1 to 0.1, from <0.1 to 0.01, from <0.01 to 0.001, and <0.001. This classification scheme categorizes the range of RBA values into the seven orders of magnitude reported for ER binding substances (**Appendix D**). Substances that tested negative (i.e., no RBA value could be calculated) were classified as negative for that test. In situations where both positive and negative test results were obtained for the same substance using the same *in vitro* ER binding assay, the substance was classified as equivocal within the RBA value category for the positive assay(s). The RBA value category obtained for a substance tested in any *in vitro* ER binding assay other than the RUC assay was then compared and classified as higher, the same, lower, or negative in relation to the RBA value category obtained for that substance in the RUC assay. The results were then inspected to identify assays that appeared to have performed (1) better than, (2) as well as, or (3) not as well as the RUC assay. Improved performance for an assay would be demonstrated by a shift in the RBA values for substances tested in common to higher RBA value categories and to having fewer negative calls, compared to the RUC assay. Equal performance would be demonstrated by both the RUC and the assay being considered having the same RBA value categories for the majority of substances tested in common. Decreased performance for an assay would be demonstrated by a shift in the RBA values tested in common to lower RBA value categories and to having more negative calls, compared to the RUC assay. The results of this approach are summarized in **Table 6-6**.

This qualitative assessment is confounded by a number of limitations, including:

· The lack of multiple test data within an assay for the majority of the substances considered;

- The lack of a common set of substance to compare across all assays;
- The limited number of substances tested in common between the RUC and any other assay;
- The assumption that each test was conducted appropriately and that all test results were accurate for that assay;
- The arbitrariness of the RBA value categories and the possible adverse effect substances with RUC RBA values near the boundary between any two RBA value categories have on the assessment; and
- The inherent complexity added to an assessment when equivocal test substances (i.e., those with multiple, discordant test results) are classified as positive only.

Despite the limitations, the assessment suggests that:

- The hERα, hERα-FP, hERβ, and rERβ assays performed better than the RUC assay, as demonstrated by a shift among the substances tested toward higher category RBA values.
- The GST-ERdef assays, except for GST-rtERdef, did not perform as well as the RUC assay, as demonstrated by a shift among the substances tested toward lower category RBA values and more substances classified as negative. Many of the negative tests were for substances classified as equivocal in the RUC assay and tested only once in the GST-ERdef assays, potentially limiting the validity of this conclusion. The GST-rtERdef assay performed as well as the human and rat ERα/β assays.
- The MCF-7 cell assay did not perform as well as the RUC assay (increased numbers of substances with lower RBA value categories/negative results), while the MCF-7 cytosol assay performed about the same as the RUC assay.
- For the two other animal based test methods, the MUC assay performed better than and the RBC not as well as the RUC assay.

Table 6-6 Qualitative Assessment of the Ability of Different ER Binding Assays to Detect Substances with Different Relative Binding Affinities (RBA Values) Compared to the RUC Assay

Assay	Result	RBA Value Range								Totals
		≥100	<100-10	<10-1	<1-0.1	<0.1-0.01	<0.01-0.001	<0.001	Negative	
RUC (97)[a]	+	6	17	6	13	16	8	3	0	
	+/-	0	0	0	1	1	4	7	0	
	-								15	
hERα (48)	Higher	-	2	1	4	7	3	2	2	21
	same	3	4	1	4	6	0	0	2	20
	lower	1	1	0	3	0	0	-	-	5
	negative	0	0	0	0	1	0	1	-	2
hERα-FP (24)	Higher	-	0	1	2	4	2	1	1	11
	same	1	1	1	1	2	0	0	2	8
	lower	1	1	0	0	1	0	-	-	3
	negative	0	0	0	0	0	1	1	-	2
hERβ (32)	Higher	-	2	1	4	7	3	2	2	19
	same	3	4	1	4	6	0	0	2	9
	lower	1	1	0	3	0	0	-	-	4
	negative	0	0	0	0	1	0	0	-	0
rERβ (24)	Higher	-	2	1	3	2	1	0	0	9
	same	3	4	1	2	0	0	0	2	12
	lower	0	0	0	0	0	0	-	-	0
	negative	0	0	0	0	1	0	1	-	2
GST-aERdef (28)	Higher	-	0	1	3	2	1	0	1	8
	same	3	5	1	1	1	0	0	1	12
	lower	0	0	0	1	0	0	-	-	1
	negative	0	0	0	0	1	2	4	-	7
GST-cERdef (27)	Higher	-	0	1	2	0	0	0	1	4
	same	3	5	1	2	3	1	0	1	16
	lower	0	0	0	0	1	0	-	-	1
	negative	0	0	0	0	0	2	4	-	6

Assay	Result	RBA Value Range								Totals
		≥100	<100-10	<10-1	<1-0.1	<0.1-0.01	<0.01-0.001	<0.001	Negative	
GST-hERαdef (28)	Higher	-	0	1	3	0	0	0	0	4
	same	2	4	0	2	1	0	0	2	11
	lower	1	1	1	0	2	0	-	-	5
	negative	0	0	0	0	1	3	4	-	8
GST-mERαdef (27)	Higher	-	0	1	2	0	0	0	0	3
	same	2	5	0	2	1	0	0	2	12
	lower	1	0	1	0	3	0	-	-	5
	negative	0	0	0	0	0	3	4	-	7
GST-rtERdef (29)	Higher	-	2	1	4	2	2	3	2	16
	same	3	3	0	1	2	0	0	1	10
	lower	0	0	1	0	0	0	-	-	1
	negative	0	0	0	0	0	1	1	-	2
MCF-7 cells (21)	Higher	-	0	0	0	0	1	0	2	3
	same	1	2	1	1	2	0	0	0	7
	lower	3	5	2	0	1	0	-	-	11
	negative	0	0	0	0	0	0	0	-	0
MCF-7 cytosol (31)	Higher	-	0	2	3	1	2	0	0	8
	same	4	10	3	0	1	0	0	0	18
	lower	0	2	0	1	1	0	-	-	4
	negative	0	0	0	0	0	1	0	-	1
MUC (24)	Higher	-	3	0	2	2	3	1	0	11
	same	1	1	1	1	4	0	0	1	9
	lower	1	0	0	2	0	0	-	-	3
	negative	0	0	0	0	1	0	0	-	1
RBC (22)	Higher	-	1	0	1	0	0	0	0	2
	same	3	3	2	0	0	1	0	0	9
	lower	1	0	1	0	3	0	-	-	7
	negative	0	0	0	0	1	3	2	-	4

[a]Number of substances.

Assessment based on substances tested in the RUC assay and at least one other *in vitro* ER binding assay**.** Data for the RUC assay entered as the number of positive (+), equivocal (+/-) (i.e., the substance was tested in more than one test with both positive and negative results obtained), and negative (-) calls for substances tested in that assay. Higher, the same, lower, and negative results signifies the occurrence of a higher, the same, lower, or negative RBA values compared to the corresponding RBA value obtained in the RUC assay for the same substance. Negative test method results in which the highest dose tested was <100 μM were not included in this assessment.

6.4 Performance of *In Vitro* ER Binding Assays

The *in vitro* ER binding assays that are the most useful as a screen for endocrine disruptors are those that are the most sensitive (i.e., have the greatest ability to detect weak ER-binding substances) and the most reliable (i.e., exhibit the lowest variance) (see **Section 7**). In addition, it might be anticipated that those assays that use ER derived from the species of interest (e.g., human for predicting human-related effects, wildlife species for predicting effects in wildlife) might be the most informative. Finally, when taking animal welfare and human health and safety issues into consideration, assays that do not use ER obtained from experimental animals or ones that do not use radioactivity, respectively, might be of the greatest utility.

The results of the quantitative and qualitative assessments of the performance of the 14 *in vitro* ER binding assays evaluated in this BRD, as well as the results of an assessment of the utility (source of ER, absence of animal use, absence of the use of radioactivity) of the various assays, are summarized in **Table 6-7**. Based on these assessments, the hERα, hERα-FP, hERβ, and GST-rtERdef assays appear to offer the greatest overall performance and utility as screening assays. The receptor used in the GST-rtERdef assay is derived from the rainbow trout and thus might be less relevant for the screening of substances that might affect endocrine function in humans. However, this assay might have greater utility in screening for ED substances that might impact wildlife. The relative utility of ERα versus ERβ assays in a screening paradigm needs further consideration. Among the substances tested in both the assays, 55% produced a higher RBA value in a hERβ assay, while 24% produced a higher RBA value in a hERα assay. This suggests that a hERβ assay might perform better in a screening battery. As another consideration, the ERα protein predominates in the uterus, while the ERβ protein is predominant in the prostate gland (Kuiper et al., 1997). Thus, inclusion of both types of estrogen receptors in a screening battery might be advantageous. However, among the 82 substances tested in common between the two assays, only two substances were discordant (i.e., one test substance was positive in a hERα assay but negative in a hERβ assay, and vice-versa), suggesting that either assay would perform equally well in a screening battery.

Table 6-7 Summary of *In Vitro* ER Binding Assay Performance

Assay	Quantitative Peformance[a]	Qualitative Performance[b]	Use of Experimental Animals[c]	ER from Species of Interest[d]	Non-radioactive Technology[e]
RUC	0				
hERα	0	+	+	+	
hERα-FP	0	+	+	+	+
hERβ	0	+	+	+	
rERβ	0	+	+		
GST-aERdef	0	-	+		
GST-cERdef	0	-	+		
GST-hERαdef	0	-	+	+	
GST-mERαdef	0	-	+		
GST-rtERdef	0	+	+	+	
MCF-7 cells	0	-	+		
MCF-7 cytosol	0	0	+		
MUC	0	+			
RBC	0	-			

[a]The quantitative assessment did not convincingly indicate that any single assay performed better than any other assay

[b]The RUC assay was used as the standard assay in the qualitative assessment; + = assays with improved performance; 0 = assays with similar performance; - = assays with lower performance than the RUC assay.

[c]Utility (+) based on the lack of need for experimental animals.

[d]Utility (+) based on the use of ER from a species of direct interest (i.e., human ER for human health, a wildlife species for ecological effects).

[e]Utility (+) based on the use of non-radioactive technology.

6.5 General Strengths and Limitations of *In Vitro* ER Binding Assays

Competitive binding assays indicate whether a substance can interact with the target receptor by its ability to displace the natural ligand. These assays do not provide sufficient evidence to conclude that a substance is an agonist or an antagonist, or take into consideration other mechanisms of action that may lead to endocrine disruption (Zacharewski, 1998). However, *in vitro* binding assays can be important components of a battery of tests and are suitable for screening, because they:

- Are cost-effective;
- Are rapid and relatively easy to perform;

- Are based on a easily quantitated, well-elucidated mechanism of action (i.e., binding to a specific protein);
- Are sensitive (50 fmol ER/mg protein can be detected);
- Can be performed using small amounts of test substances;
- Can be used to test multiple substances simultaneously; and
- Can be easily standardized among laboratories.

These assays have limitations also, including:

- Inability to distinguish agonists from antagonists; and
- Potential generation of false positive and false negative results.

In terms of false positive results, the substance might disrupt the binding of the radioactive ligand to the ER by deactivating the receptor or decrease binding via noncompetitive inhibition (Kupfer, 1988). The latter might occur at high concentrations of the test substance. For false negative results, the accurate measurement of rapidly dissociating, low affinity ligands can be difficult because the bound ER and ligand are not in equilibrium when the unbound ligand is washed away from the receptor. Under these conditions, low affinity ligands are more likely to dissociate from the ER. This dissociation is a concern when the receptor or ligand is bound to a solid support such as charcoal that is used in traditional competitive ER binding assays (National Academy of Sciences, 1999). Assays that use FP to assess ER changes would not be affected by this concern. Other mechanisms for obtaining a false negative response include metabolic activation of the test substance to an active intermediate, which subsequently binds to the ER, incomplete solubility in the assay buffer, or incompatibility with assay conditions. Because traditional ER binding assays do not include the enzymes and co-factors required for metabolic activation, some potential ER binding substances will be missed. A possible solution to this limitation is to develop *in vitro* ER binding assays that include a metabolic activation system, as has been conducted in some ER TA assays (Charles et al., 2000; Sumida et al., 2001).

6.6 Conclusions and Recommendations

Although a large number of substances have been tested in *in vitro* ER binding assays, relatively few substances have been tested more than once in the same assay or in multiple assays.

Furthermore, as the primary focus of many of the investigations using *in vitro* ER binding assays has been at understanding mechanisms of binding and transcriptional activation and not at identifying substances with ER binding activity, much of the published data are of limited value in terms of an analysis of performance. Although these limitations weaken the validity of any assessment of *in vitro* ER binding assays, some general conclusions can be made.

The quantitative assessment of lnRBA and $lnIC_{50}$ values determined that the effect of substances on the variation in RBA and IC_{50} values was much greater than the effect of assay type, and that significant differences in performance among the different *in vitro* ER binding assays were not present. One limitation of the quantitative assessment was that this approach does not consider situations in which a substance was classified as negative and positive in different tests using the same assay. The qualitative assessment considered whether RBA values (single or median) obtained for substances tested in each of 13 assays were within the same log range as the corresponding values obtained for the same substances in the RUC assay, and whether substances reported as positive or negative in the RUC assay were classified as negative or positive, respectively, in other assays. The RUC assay was selected as the assay for comparison because it had the largest database with respect to the number of substances tested and the number of laboratories using the procedure. The explicit assumption in this assessment was that an assay would perform as well as or better than the RUC assay if it demonstrated similar or higher RBA values and had the same or fewer negative calls for the same set of substances, respectively. Using this approach, the hERα/hERα-FP, hERβ/rERβ, GST-rtERdef, and the MUC assays appear to have performed better than the RUC assay, while the MCF-7 cytosol assay appears to have performed about as well as the RUC assay. The remaining eight assays did not perform as well as the RUC assay but this may reflect the level of usage and the types of substances tested rather than a lack of performance. Similar to the quantitative assessment, this approach is limited by the lack of multiple test data within an assay for most of the substances considered, and by the lack of a common substance database to compare across all assays. The assessment also assumes that each test was conducted appropriately and that the test results were accurate.

Taking into account the available *in vitro* ER binding assay database and the various quantitative and qualitative assessments conducted on the 14 *in vitro* ER binding assays considered in this BRD, the following recommendations can be made in regard to the use of such assays as screening test methods within a battery of Tier 1 endocrine disruptor tests.

- Based on a consideration of such factors as relative performance, elimination of animal use, the use of the ER from the species of interest, and the use of alternatives to radioactive substances, the hERα, hERα-FP, and hERβ assays should have the highest priority for validation as screening assays for human health-related issues, while the GST-rtERdef assay might be preferred when screening for substances that pose a hazard to wildlife. Due to an inability to conduct an adequate assessment of assay reliability (see **Section 7**), reliability was not considered in making these recommendations. However, it might be expected that assays which use semi-purified or purified ER proteins would be more reliable than those based on extracts of ER from animal tissues.
- In conducting future validation studies with these assays, the RUC assay should be used as the reference test method. The RUC assay is currently undergoing validation efforts sponsored by the U.S. EPA and the resulting performance and reliability information could be used to establish minimal performance standards for other assays.
- Formal validation studies should be conducted using appropriate substances covering the range of expected RBA values to adequately demonstrate the performance characteristics of the *in vitro* ER binding assays recommended as possible screening assays. A list of potential test substances for use in such a validation effort is provided in **Section 12**.
- There is little information about the ER binding activity of metabolites of xenobiotics and it is not clear whether metabolic activation needs to be included in *in vitro* ER binding test methods used as screening assay. This issue should be considered prior to the implementation of future validation studies.

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7.0 *IN VITRO* ER BINDING TEST METHOD RELIABILITY ASSESSMENT

7.1 Introduction

The ICCVAM Submission Guidelines (ICCVAM, 1999) request information about the assessment of test method reliability[1]. This includes a rationale for selecting the substances used to evaluate intra- and inter-laboratory reproducibility, discussion of the extent to which the substances tested represent the range of possible test outcomes, and a statistical analysis of intra- and inter-laboratory reproducibility. In addition, measures of central tendency and variation for historical negative and positive control data and an assessment of the historical control variability should be conducted.

However, no formal validation studies to assess *in vitro* ER binding assay inter- and intra-laboratory reproducibility have been conducted, and the nature of the current database for these assays precludes a formal analysis. Historically, investigators have used these assays primarily to gain insight into the mechanisms of the binding of a ligand to the ER, to compare the relative binding of different ligands to ER isolated from different tissues and/or species, and to understand the process of ER-induced TA. Only relatively recently have ER studies been conducted to investigate the biological activities of putative endocrine disruptors.

Despite these limitations, a quantitative assessment of IC_{50} and RBA values was conducted to assess the interlaboratory reproducibility of each of the 14 *in vitro* ER binding assays considered in this BRD. The assessment was based on the 238 substances tested in at least two assays (**Appendix E**), and was limited to individual tests that resulted in an IC_{50} or RBA value (i.e., the substance was classified as positive).

7.2 Quantitative Assessments of Interlaboratory Reproducibility

To reduce the extent of skewness in the data prior to conducting the quantitative assessments, the two outcome variables for *in vitro* ER binding assays -- the RBA and the IC_{50} values -- were

[1] Reliability is a measure of the degree to which a test can be performed reproducibly within and among laboratories over time, where reproducibility is the variability between single test results obtained in a single laboratory (intralaboratory reproducibility) or in different laboratories (interlaboratory reproducibility) using the same protocol.

transformed using the natural log. Studies that did not result in an IC_{50} and/or RBA value were excluded from consideration. Estimates of variance were compared across substances within the same assay, across substances without regard to the assay, and across assays without regard to the substances. A comparison of variances provides insight into which assays are the most reliable (i.e., all other aspects being equal, the smaller the variance, the more reliable the assay). Given the large number of data points for modeling, the general linear models (GLM) used in this analysis are robust, although some skewness may yet exist with the data. To simplify the comparison, each literature citation was considered an independent assessment (designated here as a 'reference').

As described in **Section 6**, two-way and three-way analysis of variance models were performed with random effects to estimate the intra-class correlation of substances. A high correlation value indicates that the lnRBA or $lnIC_{50}$ values are more similar within groups than among groups, where groups can be defined by assay or by reference. Estimates of variance for each model component and intra-class correlation are presented to show which factors (substance, assay, or reference) are responsible for the greatest variation in the lnRBA and $lnIC_{50}$ values. Due to limitations in the database with regard to the number of substances tested in multiple assays and to the number of independent tests performed for a substance using the same assay, the results of these analyses must be viewed with caution.

Initially, all data representing all substances, assays, and references were considered, and unique data (i.e., substances tested only in a single assay) were excluded from subsequent analyses. Information on the distribution of lnRBA and $lnIC_{50}$ values, as a function of data points, assays, and references are provided in **Section 6.2.** Consistent with the quantitative analysis on performance, the $lnIC_{50}$ and the lnRBA values for 17β-estradiol were omitted from these analyses.

7.2.1 Measures of Intra-Class Correlation

The intra-class correlation, r_I, measures the percentage of variation in y, the outcome variable, explained by a given component or set of components. The model is y = substance + assay + reference. **Table 7-1** contains the components of variance for each variable adjusted for the

other two variables (see also **Section 6**). Interpretation of this analysis is limited to factors that impact on reliability; factors that impact on assay performance are discussed in **Section 6**. From this analysis, it appears that the lnRBA or $lnIC_{50}$ values calculated for a specific substance were generally consistent irrespective of how many times a substance was tested using the same assay.

Table 7-1 Components of Variance for Each Variable Adjusted for the Other Two Variables – Reliability Assessment

	Outcome, y (% variation)	
	lnRBA	$lnIC_{50}$
Var(substance)	8.34	8.49
Var(assay)	0.38	0.34
Var(reference)	1.40	2.01
Var(error)	1.75	2.44
Corr (y_{ijk}, $y_{ij'k'}$)	0.70	0.64
Corr (y_{ijk}, $y_{ijk'}$)*	0.73	0.67
Corr (y_{ijk}, $y_{ij'k}$)	0.82	0.79

*A high correlation was found for a substance tested in the same assay (i.e., the variation in response of a substance within an assay was similar to that observed across assays).

The high correlation suggests that little variation existed in test results for an individual substance tested multiple times. However, because the majority of repeat tests were conducted on substances that were relatively potent in terms of *in vitro* ER binding, it is not known if similar variances would be found among weakly binding substances.

7.2.2 Evaluation of Substances Tested in Nine or More *In Vitro* ER Binding Assays

In this analysis, the variances for the RBA values of 12 substances that had been tested in at least 9 of the 14 *in vitro* ER binding assays were determined. The variances and sample sizes for these 12 substances are provided in **Table 7-2,** ranked in descending order according to the median RBA value based on all positive test data. Only assays for which could be calculated are included, and most of these variances were based on three or four values only. Due to the lack of sufficient data, a corresponding analysis of IC_{50} values was not conducted.

Table 7-2 Variance of lnRBA by Substance and Assay – Reliability Assessment[a]

Substance[b] (CASRN)	Median[c] RBA	#of Obs/ # Assays	hERα[d]	hERα-FP[d]	hERβ[d]	MCF-7 cytosol[d]	MUC[d]	RUC[d]	p1*	p2**
4-Hydroxy-tamoxifen (68047-06-3)	168	18/13	0.28 (3)	1.82 (3)					0.08	0.15
DES (56-53-1)	127	38/14	0.99 (3)	0.45 (4)			0.60 (7)	3.62 (11)	0.15	0.99
Estrone (53-16-7)	45	18/13				2.40 (3)		0.98 (4)	0.73	na[e]
Estriol (50-27-1)	15.8	16/12				2.42 (4)			0.53	0.64
Zearalenone (17924-92-4)	15.0	11/9	All n≤2						0.42	na
Tamoxifen (10540-29-1)	5.0	21/14	0.44 (3)					2.95 (4)	0.02	0.10
Coumestrol (479-13-0)	3.1	15/11	0.79 (3)						0.02	0.25
HPTE (2971-36-0)	1.45	12/10						1.53 (3)	0.82	na
Genistein (446-72-0)	1.30	18/11	1.07 (4)		0.97 (3)				0.11	0. 18
Bisphenol A (80-05-7)	0.031	22/14	1.36 (3)					1.25 (5)	1.25	0.60
o,p'-DDT (789-02-6)	0.038	17/12						2.97 (5)		
Kepone (143-50-0)	0.027	11/9						1.39 (3)	0.60	na

[a]Only assays where a variance could be calculated for at least one of the 12 substances are listed. The variance for a particular assay could be calculated only if a particular substance was tested three or more times in that assay; empty cells indicate insufficient data to calculate a variance. The p values could be calculated only if there were two observations from at least three or more assays; a missing p-value indicates insufficient data.

[b]Substances that had been tested in at least nine of the 14 *in vitro* ER binding assays; DES = diethylstilbestrol; *o,p'*-DDT = *o,p'*-dichlorodiphenyltrichloroethane; HPTE = (2,2-Bis(*p*-hydroxyphenyl)-1,1,1,-trichloroethane

[c]The median RBA value across assays, based on positive test data.

[d]The numbers in parenthesis indicate the numbers of replicate tests.

[e]na = No p value could be calculated since there was either no values or only one value per assay x response combination.

*p1 tests whether there is a significant difference among all assays used; unadjusted for references.

**p2 tests whether there is a significant difference among all assays used; adjusted for references.

The similarity between the p1 and p2 values for most of these substances suggests that there were no significant differences in the performance of the assays by different laboratories (a measure of assay reliability). However, DES and coumestrol exhibited considerably less variability when the analysis was adjusted for the reference (i.e., p2 is much greater than p1), suggesting that laboratory-specific differences in testing of this substance were responsible.

7.2.3 Variability in Standard Deviation for lnRBA and $lnIC_{50}$ Values by *In Vitro* ER Binding Assay

Because of insufficient data on substances tested in the same assay within or across laboratories, separate correlations between pairs of the 14 assays were not calculated. However, standard deviations of the mean of the lnRBA and the $lnIC_{50}$ values were inspected to see which assays have the least or the most variability in their responses (**Table 7-3**). A major limitation of this analysis is that the same substances were not tested in each assay. An additional limitation is the varied number of substances tested more than once in each assay. These limitations will affect any interpretation of the results. The assays in **Table 7-3** are sorted in descending order based on the number of different substances tested in each assay.

Not unexpectedly, for the same set of substances, there appears to be more variability in the IC_{50} values than in the corresponding normalized RBA values. The standard deviations for the majority of substances clustered around a median of 3.23 for the lnRBA values and 3.52 for the $lnIC_{50}$ values. The standard deviations for the lnRBA values vary from a low of 2.92 for the rERβ assay to a high of 5.09 for the RBC assay, while the corresponding standard deviations for the $lnIC_{50}$ values vary from a low of 2.95 for the GST-hERα assay to a high of 4.85 for the RBC assay. The MCF-7 cells, hERβ, GST-hERα and MCF-7 cytosol assays exhibited similar and relatively lower standard deviations for lnRBA values, while the GST-aERdef, hERβ and GST-rtERdef assays exhibited similar and relatively lower standard deviations for $lnIC_{50}$ values. The RBC assay exhibited the largest standard deviation for both the lnRBA and $lnIC_{50}$ values. Based on this analysis, the hERβ assay appears to be the most reliable, while the RBC assay appears to be the least reliable. However, these conclusions must take into account the number of substances that have been tested in each ER assay and, although not specified, the number of

laboratories that generated the data. In general, the standard deviation increases as the number of substances tested in an assay increases or as more laboratories are involved.

Table 7-3 Standard Deviation for lnRBA and $lnIC_{50}$ Values for *In Vitro* ER Binding Assays

Assay	Number of Different Substances	LnRBA		$lnIC_{50}$	
		Standard Deviation	n[a]	Standard Deviation	n[a]
RUC	100	4.34	164	4.30	90
hERα	87	3.26	112	3.69	24
hERβ	74	3.03	91	3.24	30
MCF-7 cytosol	63	3.07	72	3.55	15
MCF-7 cells	58	2.94	49	3.46	2
GST-rtERdef	43	3.20	43	3.26	43
MUC	33	3.49	49	3.37	35
GST-hERαdef	29	3.01	29	2.95	29
rERβ	28	2.92	28	-	0
GST-aERdef	25	3.19	25	3.15	25
GST-cERdef	21	3.68	21	3.68	21
RBC	21	5.09	22	4.85	8
GST-mERαdef	19	3.53	19	3.52	19
hERα-FP	19	3.84	28	3.80	28

[a]Total number of data points considered in the analysis.

7.2.4 Variability in the IC_{50} for 17β-Estradiol

The most extensive database within and across assays is for 17β-estradiol, the natural estrogen commonly used as the reference substance in *in vitro* ER binding assays for calculating the RBA value of a test substance. However, because the RBA value for this substance is arbitrarily set at 100, this measure of binding cannot be analyzed for variability. In contrast, an analysis of the IC_{50} values of 17β-estradiol, where reported, provides a means for assessing assay reproducibility. Fifty-eight IC_{50} values were available for 17β-estradiol in the ER binding database (**Appendix D**). The variability in the natural log of IC_{50} values of 17β-estradiol was compared across assays. As the sample size within each assay is quite small, only descriptive statistics of this parameter are presented (**Table 7-4**). The IC_{50} values are sorted in descending order based on the number of times 17β-estradiol was tested in each assay.

Table 7-4 Standard Deviation for IC_{50} Values Obtained for 17β-Estradiol

Assay	N[a]	Standard Deviation[b]
RUC	13	0.90
MUC	10	2.21
hERα	9	0.99
hERα-FP	7	0.61
hERβ	4	0.78
MCF-7 cytosol	3	4.06
GST-hERαdef	3	0.44
GST-rtERdef	2	0.044
GST-aERdef	2	0.15
GST-cERdef	1	
GST-mERαdef	1	
MCF-7 cells	1	
RBC	1	
rERβ	1	

[a]Number of data points considered.
[b]Standard deviations could not be calculated for single test data.

In this analysis, the greater the standard deviation, the less reliable is the assay. Since the IC_{50} values of 17β-estradiol for the rERβ, RBC, MCF-7 cells, GST-mERαdef, and GST-cERdef assays were reported by one laboratory only, no standard deviations could be calculated. Although the standard deviations for the IC_{50} values were very small for the GST-aERdef, GST-rtERdef, and GST-hERαdef assays, only two or three data points were reported for these assays. Among the assays with at least six data points (hERα-FP, MUC, RUC, hERα), the standard deviations in the $lnIC_{50}$ values are generally similar except for the hERα-FP assay where the value is smaller. Although this decreased standard deviation suggests that the hERα-FP assay is the most reliable of these four assays, the hERα-FP assay data were generated by fewer laboratories, which may have impacted on the extent of variability.

7.3 Reliability of *In Vitro* ER Binding Assays

The *in vitro* ER binding assays that are the most useful as a screen for endocrine disruptors are those that are the most sensitive (i.e., have the greatest ability to detect weak ER-binding substances) (see **Section 6**) and the most reliable (i.e., exhibit the lowest variance). The results

of the quantitative assessments of the comparative reliability of the 14 *in vitro* ER binding assays evaluated in this BRD are summarized in **Table 7-5**.

Table 7-5 Summary of *In Vitro* ER Binding Assay Reliability

Assay	lnRBA[a]	$lnIC_{50}$[a]	IC_{50} 17β-Estradiol[c]
RUC	-	-	0
GST-aERdef	0	+	?
GST-cERdef	-	0	?
GST-hERαdef	+	+	?
GST-mERαdef	0	0	?
GST-rtERdef	0	+	?
hERα	0	0	0
hERα-FP	-	-	+
hERβ	+	+	?
MCF-7 cells	+	0	?
MCF-7 cytosol	+	0	?
MUC	0	+	0
RBC	-	-	?
rERβ	+	?	?

[a]Reliability based on standard error term for lnRBA values (**Table 7-3**); more reliable = +; average reliability = 0; less reliable = -.

[a]Reliability based on standard error term for $lnIC_{50}$ values (**Table 7-3**); more reliable = +; average reliability = 0; less reliable = -; ? = the number of observations was too small to make a determination.

[c]Reliability based on variance analysis of $lnIC_{50}$ values for 17β-estradiol (**Table 7-4**); most reliable = +, average reliability = 0; least reliable = -; ? = the number of observations was too small to make a useful determination.

Based on a weight-of-evidence approach, the GST-hERαdef and hERβ assays appear to offer the greatest overall reliability (both assays had two reliable categories). However, due to the absence of formal validation studies to assess reliability and to the paucity of the data on which this reliability assessment is made, the decision to select any one assay or group of assays over another appears to be arbitrary.

7.4 Conclusions and Recommendations

Although a large number of substances have been tested in *in vitro* ER binding assays, relatively few substances have been tested more than once in the same assay or in multiple assays, and no

formal validation studies have been conducted to assess reliability. A quantitative assessment was conducted using the available IC_{50} and RBA data after being log-normal transformed to reduce possible skewness. One limitation of this approach was that situations in which a substance was classified as negative and positive in different tests using the same assay was not considered.

An analysis of the variances for the RBA values of 12 substances that had been tested in at least nine of the 14 *in vitro* ER binding assays suggested that there were no significant differences in the reliability of the assays as performed by different laboratories. Inspection of the standard errors of the mean of the lnRBA and the $lnIC_{50}$ values suggested that the RUC assay appeared to be the most reliable, while the RBC assay appeared to be the least reliable. A major limitation of this analysis is that the same substances were not tested in each assay and that the number of substances that have been tested in each ER assay or the number of laboratories that generated the data was not considered.

A comparison of the variability in lnRBA and $lnIC_{50}$ values across assays, ignoring substance effects, indicated that the GST-hERαdef and hERβ assays were the most consistent and the RBC assay was the least consistent among the 14 assays evaluated. An analysis of the variability in the $lnIC_{50}$ for 17β-estradiol, the reference estrogen for these assays, indicated that the most consistent results were obtained with the hERα-FP assay, while the MUC, RUC, and hERα assays exhibited somewhat greater but comparable variances. The low variability associated with the hERα-FP assay, however, might be a reflection of the small number of laboratories that have reported RBA values using this method. Data were too limited to evaluate the other *in vitro* ER binding assays.

Taking into account the available *in vitro* ER binding assay database and the various quantitative assessments conducted on the 14 *in vitro* ER binding assays considered in this BRD, the following recommendation can be made in regard to the use of such assays as screening test methods within a battery of Tier 1 endocrine disruptor tests.

- Despite inferences that the GST-hERαdef and hERβ assays appear to be the most reliable among the 14 *in vitro* ER binding assays considered in this BRD, an adequate assessment of

assay reliability cannot be performed based on the limited database available. However, it might be expected that assays that use semi-purified or purified ER proteins would be more reliable than those based on extracts of ER from animal tissues.

- It is essential that validation studies be conducted to assess assay reliability and that these validation studies use appropriate substances covering the range of expected RBA values. A list of potential test substances for use in such a validation effort is provided in **Section 12**.

8.0 QUALITY OF DATA REVIEWED

8.1 Extent of Adherence to GLP Guidelines

Ideally, all data supporting the validity of a test method should be obtained and reported in accordance with GLP guidelines, which are nationally and internationally recognized rules designed to produce high-quality laboratory records. GLPs provide a standardized approach to report and archive laboratory data and records, and information about the test protocol, to ensure the integrity, reliability, and accountability of a study (U.S. EPA, 2001, 2002; FDA, 2002).

Based on the available information, none of the published *in vitro* ER binding studies identified for this BRD appear to have been conducted in compliance with national or international GLP guidelines.

8.2 Assessment of Data Quality

Formal assessments of data quality, such as a quality assurance (QA) audit, generally involve a systematic and critical comparison of the data provided in a study report or published paper to the laboratory records generated for a study. No attempt was made to formally assess the quality of the *in vitro* ER binding data included in this BRD. The published data on the competitive binding of substances to the ER were limited to RBA and, to a lesser extent, IC_{50} and K_i values. Auditing these reported values would require obtaining the original data for each ER binding experiment, which is not readily available.

An informal assessment of the ER binding publications revealed limitations that complicate interpretation of the *in vitro* ER binding assay data (**Appendix D**):

- *Insufficient methods information*: A relatively large number of publications contained limited details about the methods used to conduct the studies. In some cases, publications reported that the methods were "performed as previously described," and in many of these cases the cited publication referenced another publication for experimental details. Following this trail of references made it difficult to determine the actual protocol used to produce the data reported in the publication being abstracted.
- *Inconsistent nomenclature of test substances*: Most publications did not provide CASRNs for the substances tested, which in some cases made an unequivocal identification difficult. For

example, 19 publications reported results for a hydroxylated form of tamoxifen. Most laboratories reported testing "4-hydroxytamoxifen"; however, a few publications used less specific substance names, such as "monohydroxytamoxifen" and "hydroxytamoxifen," which do not specify the location of the hydroxy group on the parent molecule. As a result, it is not possible to conclude definitively that these three names referred to same substance.

- *Data reporting*: A few publications calculated the RBA value of a test substance using the IC_{50} value of 17β-estradiol reported in another publication. Thus, it could not be determined whether the test substance and 17β-estradiol were evaluated concurrently in the same experiment. Additionally, much of the data reported in the publications were RBA values only, with no accompanying error term provided to assess the quality of the estimate. Thus, the variability of the experimental data could not be assessed.
- *High number of unreplicated studies*: A majority of the substances tested in ER binding studies have not been tested in multiple laboratories, and thus, the results are unconfirmed. Of the 638 substances included in this BRD, 376 (59%) were tested by one laboratory only.
- *Graphical presentation of data*: Some publications presented the results of ER binding experiments in graphical format only. A majority of these publications presented IC_x data in a semi-log plot (e.g., % [^{3}H]17β-estradiol vs. log concentration of competitor). In these cases, IC_{50} values were estimated from the graphs, and used to calculate the corresponding RBA values. These estimations might contribute to some of the variability seen in the RBA values in **Appendix D**.

8.3 Quality Control Audit

NICEATM staff conducted a quality control (QC) audit of the ER binding database provided in **Appendix D**. In conducting this audit, data input into the database was checked against the original sources and corrected if an entry error had been made.

8.4 Need for Data Quality

Data quality is a critical component of the test method validation process. To ensure data quality, ICCVAM recommends that all of the data supporting validation of a test method be available with the detailed protocol under which the data were produced. Original data should be

available for examination, as should supporting documentation, such as laboratory notebooks. Ideally, the data should adhere to national or international GLP guidelines (ICCVAM, 1997).

All of the *in vitro* ER binding assay data included in this BRD were obtained from peer-reviewed scientific articles reporting the results of studies conducted at facilities that do not typically perform studies in compliance with GLP guidelines. It should be noted that a majority of these studies were performed in response to basic research questions and/or to evaluate the binding affinities of estrogen analogs or new drugs, not to support prevalidation or validation of the test method, or the formal submission of data to regulatory agencies. Because these studies span three decades and a multitude of laboratories, verifying the integrity of the data via a formal audit process was not possible.

An informal assessment of the *in vitro* ER binding assay data showed that the test substances and data were not consistently represented in the same format. In addition, the methods were presented in varying levels of detail and completeness. Since the published data were not verified for their accuracy against the original experimental data, caution must be exercised when interpreting the quantitative and qualitative analyses performed in **Section 6**.

An important step towards acceptance of *in vitro* ER binding assay methods into a regulatory screening program is production of high quality data. To achieve this goal, it is recommended that any future prevalidation and validation studies on *in vitro* ER binding assays be conducted with coded substances and in compliance with national and international GLP guidelines. Ideally, the substances should be obtained from a common source, and distributed from a central location. Laboratories not able to perform studies in compliance with GLP guidelines should perform studies in the spirit of GLP. At a minimum, this would require detailed, accurate documentation of laboratory protocols, experiment-related notes, and data entries.

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9.0 OTHER SCIENTIFIC REPORTS AND REVIEWS

9.1 Availability of Other *In Vitro* ER Binding Data

A number of the peer-reviewed publications identified during the initial literature search that contained ER binding data were not abstracted for inclusion in this BRD. These include:

- Studies lacking either appropriate quantitative data (i.e., RBA or IC_{50} values) or the necessary information to calculate IC_{50} values;
- Studies for which test substances were not adequately identified;
- Studies containing data from unique procedures (e.g., use of T47D cells or bovine uterine cytosol); and
- Publications reporting results for only a few substances that had not been tested by any other investigator.

Recognizing that unpublished ER binding data may be available, a formal request was published in the *Federal Register* (Vol. 66, No. 57, pp.16278 - 16279) for data and/or information from completed studies using or evaluating ER binding assays. No information was received in response to this request.

It is known that some companies involved in the development of pharmaceuticals routinely use *in vitro* ER binding assays to screen substances for their potential estrogenic activity. However, these data are unpublished and have not been provided to NICEATM for consideration.

The U.S. EPA has a contract with Battelle Pacific Northwest National Laboratory (Richland, WA) to generate *in vitro* ER binding data to evaluate two QSAR ER binding models developed by scientists at the U.S. Food and Drug Administration National Center for Toxicological Research (FDA NCTR) and by Dr. Ovanes Mekenyan (Mekenyan et al., 2000). Initially, Battelle will test 25 substances in an *in vitro* ER binding RUC assay. The ultimate goal is to test a total of 300 substances for evaluation in the two QSAR models, which the U.S. EPA plans to use for priority setting of substances for the U.S. EPA EDSP. In addition, the American Chemistry Council (ACC) is sponsoring *in vitro* ER binding studies, using the RUC assay, at two laboratories that will be testing approximately 25 substances. Neither the U.S. EPA nor the ACC test results are available at this time.

While every effort was made to include all available, pertinent *in vitro* ER binding assay data in this BRD, some data may have been excluded inadvertently.

9.2 Conclusions of Other Scientific Reviews of *In Vitro* ER Binding Methods

To date, no independent peer reviews of *in vitro* ER binding assays have been conducted. However, two workshops addressed the use of these assays as potential endocrine disruptor screening methods. Although the strengths and limitations of these assays were discussed at both workshops, no effort was made to evaluate the reliability or performance of these assays. Some of the conclusions from these workshops are summarized below.

9.2.1 1996 Endocrine Disruptor Screening Methods Workshop

In vitro ER binding assays were discussed extensively at an Endocrine Disruptor Screening Methods Workshop held in July 1996 at Duke University in Durham, North Carolina. Gray et al. (1997) edited the proceedings of this workshop, which was cosponsored by the U.S. EPA, the Chemical Manufacturer's Association (CMA), and the World Wildlife Fund (WWF).

The major strengths of *in vitro* cytosolic ER binding assays cited by the authors include:

- Sensitivity (can detect ER binding with as low as 50 fmol ER/mg protein);
- Specificity of response;
- Relatively short duration of the test;
- Fairly inexpensive;
- Well-documented; and
- Can be standardized.

The major limitations cited by the authors include:

- Do not distinguish between estrogen agonists and antagonists;
- Substances requiring metabolic activation would produce false negative results;
- Insolubility of test substance in assay buffer could produce a false negative result; and
- Denaturation effects of a test substance could produce false positive results.

In addition, the authors briefly discussed the major advantages and disadvantages of cell-free and whole-cell binding assays using hER. The major strength of these assays is their potential relevance to humans, while their major limitation is that they are relatively new methods with little published data.

9.2.2 1997 Workshop on Screening Methods for Detecting Potential (Anti-) Estrogenic/Androgenic Chemicals in Wildlife

In March 1997, the U.S. EPA, the CMA, and the WWF cosponsored a workshop in Kansas City, Missouri, that addressed the use of ER binding assays as screening methods for detecting potential (anti-) estrogenic chemicals in wildlife. Proceedings of this workshop were published by Ankley et al. (1998).

The major advantages cited by the authors of using ER binding assays as endocrine disruptor screens for wildlife include:

- Widespread acceptance and use; and
- Can be conducted with ER from various mammalian and nonmammalian species, including fish, reptiles and birds.

The major disadvantages include:

- Do not distinguish between agonists and antagonists; and
- Uncertainties regarding extrapolation across species.

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10.0 ANIMAL WELFARE CONSIDERATIONS

10.1 Refinement, Reduction, and Replacement Considerations

ICCVAM promotes the scientific validation and regulatory acceptance of new methods that refine, reduce, or replace animal use where scientifically feasible (ICCVAM, 1997; P.L. 106-545). Refinement, Reduction, and Replacement are known as the three Rs of animal protection. These principles of humane treatment of laboratory animals are described as:

- Refining experimental procedures such that animal suffering is minimized;
- Reducing animal use through improved science and experimental design; and
- Replacing animal models with nonanimal procedures (e.g., *in vitro* technologies), where possible.

Combes (2000) and Phillips (2000) recommended that adequate consideration be given to animal welfare concerns by careful development and validation of all proposed endocrine disruptor screening methods. With respect to the proposed use of *in vitro* ER binding assays as screening methods to detect substances that potentially exhibit estrogenic or anti-estrogenic activity, it is important to evaluate the current level of animal use in these assays and to consider what opportunities exist for refining, reducing, or replacing procedures that use animals.

10.2 Use of Animals in *In Vitro* ER Binding Assays

Of the 14 *in vitro* ER binding assays considered in this BRD, three assays (RUC, MUC, RBC) require the collection of uterine tissue from female rats, mice, or rabbits. Because the animals are not treated with a test substance, treatment-related pain and suffering are avoided. Some investigators that use the RUC and MUC assays obtain the uterus from ovariectomized mature female rats, while other investigators use nonovariectomized, sexually immature female rats. Some investigators prefer the former procedure because removal of the ovaries appears to increase uterine ER production in the rat for about 5 to 14 days after an ovariectomy. Thus, more ER can be obtained per gram of uterine tissue in comparison to the procedure using non-ovariectomized, sexually immature females. One investigator who uses uteri from overiectomized rats in the RUC assay estimates that one average-sized mature rat uterus (~200 mg) generates enough cytosol to test one substance at six concentrations in triplicate

(personal communication, Dr. Hong Fang, NCTR). Corresponding information on the amount of cytosol generated from sexually immature rats was not obtained.

With respect to refining the uterine cytosol assays, procedures that are the least invasive and distressful to the animals should be used. As for reducing the number of animals used in these assays, protocols should maximize the number of substances that can be tested per gram of tissue, for example, by optimizing the protocol to use the lowest possible concentration of ER per assay tube. In addition, the use of sexually mature versus immature animals should be carefully considered. While the use of immature animals only would reduce the need for ovariectomies, using sexually immature animals, which have substantially smaller uteri than mature ovariectomized animals (e.g., 30-50 mg versus 200 mg for the rat), would require that more animals be used.

The other 11 *in vitro* ER binding assays considered in this BRD do not use animals. Two of these assays -- the MCF-7 cell and MCF-7 cytosol assays -- use a human cell line, while the remaining assays use purified or semi-purified human or animal receptors derived from cDNA or from GST-ER fusion proteins. The experimental systems using purified receptors, semi-purified receptors, or fusion proteins can be carried out in multiwell plates, which permit smaller reaction volumes and allow data collection to be partially or fully automated. With the potential for automation, these systems would be more economical to perform than the uterine cytosol assays, which require animal care and surgical costs. Another advantage to using purified (cloned) ER is that ERα and ERβ can be used selectively.

The assays using human ER or the ligand binding domain of the human ER are directly relevant to humans, as compared to ER derived from rodent or rabbit tissues. However, because of the relative newness of these assays, they have not been used as extensively as the uterine cytosol assays for the routine testing of substances; thus, their reliability and performance have not been demonstrated to the same extent. Despite the lack of a substantial database on assays using purified and semi-purified ERs, these assays, with further development and validation, could potentially replace the use of uterine cytosol to determine the ER binding of substances.

11.0 PRACTICAL CONSIDERATIONS

11.1 Test Method Transferability

Test method transferability addresses the ability of a method to be accurately and reliably performed by multiple laboratories (ICCVAM, 1997). This definition includes laboratories experienced in the particular type of procedure, and otherwise competent laboratories with less or no experience in the particular procedure. It also addresses whether the necessary facilities, equipment, and trained staff to perform the method can be readily obtained, and whether the cost of the assay and the level of expertise or training needed are considered reasonable. The degree of transferability of a test method affects its interlaboratory reproducibility.

The ICCVAM Submission Guidelines (ICCVAM, 1999) request a discussion of test method transferability with respect to the following factors:

- Availability of the facilities and the major fixed equipment needed to perform the test method;
- The training requirements for laboratory personnel to demonstrate proficiency with the test method;
- Costs involved in conducting the test; and
- Time needed to conduct the test.

11.1.1 Facilities and Major Fixed Equipment

The facilities needed to conduct ER binding assays are widely available, and the necessary equipment is readily available from major suppliers. Specific needs as related to the various *in vitro* ER binding procedures are described below. To ensure personnel and community safety, pertinent State or Federal regulations for the handling of hazardous and radioactive substances/wastes must be strictly adhered to.

Uterine Cytosol ER Binding Assays

Facilities: Standard toxicology, biochemistry, or molecular biology laboratory supplies, and an animal facility containing temperature, humidity, and light controls. A small animal surgical facility is recommended for laboratories that prefer not to purchase ovariectomized animals from animal suppliers.

Major Fixed Equipment: Refrigerated centrifuge, ultracentrifuge, and liquid scintillation counter.

MCF-7 Cells/Cytosolic Assays and Semi-Purified ERα and ERβ or GST Fusion Proteins

Facilities: Standard cellular or molecular biology laboratory with cell culture capabilities.

Major Fixed Equipment: Liquid scintillation counter.

Purified Human ER Measured by Fluorescent Polarization

Facilities: Standard cellular or molecular biology laboratory.

Major Fixed Equipment: Fluorescence polarization instrument.

11.2 Training Considerations

Uterine Cytosol ER Binding Assays

Basic laboratory skills and training in small animal handling and surgery.

MCF-7 Cells/Cytosolic Assays

Basic laboratory skills and training in cell culture techniques.

Semi-purified ERα and ERβ

Basic laboratory skills with training in molecular biology, particularly cloning, cell culture techniques and protein purification.

GST Fusion Proteins

Basic laboratory skills with training in molecular biology, particularly cloning, bacterial cell culture techniques and protein purification.

Purified Human ER Measured by Fluorescent Polarization

Basic laboratory skills.

11.3 Cost and Time Considerations

Table 11-1 provides information on the estimated cost per sample, the expected duration of the study, special equipment needed, and other considerations. The cost information provided was obtained from scientists working at not-for-profit institutions and would be an underestimate for studies conducted at contract laboratories in compliance with GLP guidelines. Where estimated costs are not provided, it is probably safe to assume that the costs for all of the uterine cytosol assays (RUC, MUC, RBA) are roughly equivalent. Similarly, it would be expected that the costs for the assays using semi-purified ER or GST constructs and the cell culture assays would be roughly equivalent.

Table 11-1 Comparison of Costs, Time, and Special Equipment Needs of Different ER Binding Assays

Assay	Cost/ Test substance	Duration (hours)	Special Equipment	Other Considerations
RUC	$135	~24 – 48	Liquid scintillation counter ($15K - $30K)	
MUC	n.a.	~24 – 48	Liquid scintillation counter ($15K - $30K)	
RBC	n.a.	~24 – 48	Liquid scintillation counter ($15K - $30K)	
MCF-7 cytosol	n.a.	~24 – 48	Liquid scintillation counter ($15K - $30K)	
hERα	n.a.	~24	Liquid scintillation counter ($15K - $30K)	
hERα-FP	$65	~4	Fluorescence polarimeter ($20K - $35K)	No radioactive wastes. Proprietary fluorescein-labeled estrogen ligand.
hERβ	n.a.	~24	Liquid scintillation counter ($15K - $30K)	
rERβ	n.a.	~24	Liquid scintillation counter ($15K - $30K)	
GST-hERαdef	$30	~8	Liquid scintillation counter ($15K - $30K)	
GST-mERαdef	$30	~8	Liquid scintillation counter ($15K - $30K)	
GST-aERdef	$30	~8	Liquid scintillation counter ($15K - $30K)	
GST-cERdef	$30	~8	Liquid scintillation counter ($15K - $30K)	
GST-rtERdef	$30	~8	Liquid scintillation counter ($15K - $30K)	
MCF-7 cells	n.a.	~24 – 48	Liquid scintillation counter ($15K - $30K)	

n.a. = Cost estimate not available in the literature or from laboratories conducting the assay.

12.0 MINIMUM PROCEDURAL STANDARDS FOR *IN VITRO* ER BINDING ASSAYS AND RECOMMENDATION OF SUBSTANCES FOR USE IN VALIDATION STUDIES

12.1 Introduction

Although published studies on the ability of substances to bind *in vitro* to the ER are relatively numerous, there are no published standard test guidelines for conducting such studies, and no formal validation studies have been performed to assess the reliability or performance of ER binding assays. To support the further standardization and validation of *in vitro* ER binding assays, minimum procedural standards for such assays and a recommended list of test substances for use in validation studies are provided. The minimal procedural standards and recommended test substances are based on a comparative evaluation of the 14 *in vitro* ER binding assays summarized and evaluated in this BRD (**Sections 6** and **7**). The RUC assay, which has been the most widely used method for identifying substances with ER binding activity, is proposed as the standard against which new tests should be evaluated.

12.2 Minimum Procedural Standards

12.2.1 Animal Studies

All studies utilizing animals should be approved by the Institutional Animal Care and Use Committee (IACUC) or its equivalent.

12.2.2 Dissociation Constant (K_d) of the Reference Estrogen

Irrespective of the source of the ER used, the dissociation constant, K_d, of the reference estrogen (e.g., 17β-estradiol) must be determined each time the assay is performed. The purpose of determining K_d is to demonstrate that the assay system is valid (e.g., a finite number of high affinity receptors are saturated with ligand) and to optimize the system with respect to receptor and ligand concentration. The K_d is determined in a saturation binding experiment that involves adding increasing concentrations of the radiolabeled reference estrogen to the ER preparation and measuring binding to the ER (Motulsky, 1995). To calculate specific binding of the radiolabeled reference estrogen to the ER, nonspecific binding is measured at each radioligand concentration by the addition of a nonlabeled estrogen at a concentration that occupies all available receptors. The nonspecific binding is then subtracted from the total binding (in the

absence of nonlabeled compound) of the radiolabeled reference estrogen (Motulsky, 1995). The K_d of the reference estrogen, which reflects its affinity for the specific ER preparation, can then be calculated, and is used to determine the appropriate concentration of reference estrogen to be used in competitive binding assays. To determine the K_d, the ER must be exposed to the reference estrogen at concentrations spanning five to six orders of magnitude.

12.2.3 Preparation of Test Substances

Test substances must be dissolved in water or in a solvent that is miscible with water. For substances not sufficiently water soluble, absolute ethanol, or DMSO are proposed as solvents. Preference is given to ethanol since this solvent has been used in most of the studies conducted to date. Other solvents may be used as long as it can be demonstrated that they do not interact with the test system. A solvent control set of assay tubes must be included in each assay. It might be necessary to characterize the solubility of the test substance in several solvents to identify the optimal solvent to use in the ER binding assay.

12.2.4 Concentration Range of Test Substances

To minimize effort and costs in screening/testing, and in recognition that adding excessive amounts of a test substance can perturb the test system through physicochemical mechanisms, most testing schemes include a limit dose (i.e., the highest dose that should be tested in the absence of solubility constraints). An agreed upon limit dose for *in vitro* ER binding screening assays has not been established. Historically, the highest dose tested in such assays has ranged generally from 1 to 100 μM, with some tests conducted at doses as high as 1 mM. The IC_{50} values (and thus the RBA values) reported for substances tested in various *in vitro* ER binding assays cover six orders of magnitude below the IC_{50} for 17β-estradiol, the reference estrogen. In the RUC assay, the median IC_{50} for 17β-estradiol is 3.8 nM. Thus, if testing for ER binding substances requires the ability to detect substances with an IC_{50} that is at least six orders of magnitude lower than that of 17β-estradiol, then the limit dose (unless precluded by chemical properties such as solubility) should be above 4 mM (e.g., 10 mM) to allow for the detection of an IC_{50} in the concentration range of interest. However, if five orders of magnitude are sufficient for RBA values, then the limit dose would have to be above 400 μM (e.g., 1 mM). Decreasing

the limit dose to 100 μM would limit the sensitivity of the assay to RBA values that cover approximately four orders of magnitude.

For the purpose of screening, it is proposed that the limit dose be 1 mM and that a concentration range from 1 mM to 1 nM, in 10-fold increments, be used. However, if it is suspected that the test substance may bind more strongly to the ER than 17β-estradiol, the dose range should extend from 10 pM to 10 μM in 10-fold increments.

For relatively insoluble substances, the highest dose should be at the limit of solubility and the concentrations tested should be in 10-fold increments. Testing at concentrations that precipitate in the test medium should be avoided to minimize false positive results associated with the non-specific interaction of the precipitate with the ER (Gray et al., 1997).

12.2.5 Solvent and Positive Controls

Concurrent negative, solvent, and positive controls must be included in each experiment. The negative control contains all the reagents of the test system, except the assay solvent, which is replaced with a known nonreactive material, such as water. This sample is processed with treated samples and other control samples to ensure the solvent does not interact with the test system. The solvent control consists of all the reagents of the test system, including the solvent, and should be tested at the highest concentration that is added with the test substance. A positive control substance is included to demonstrate the sensitivity of each experiment and to allow for an assessment of variability in the conduct of the assay across time. The volume of materials in the ER assay control tubes should equal that of ER assay tubes containing test substance and reference estrogen. Since the RBA for the reference estrogen, 17β-estradiol, is set at 100, it is recommended that a substance (e.g., tamoxifen, coumestrol) that induces an RBA value between two and three orders of magnitude lower be used as the positive control. The median RBA values of tamoxifen and coumestrol in the RUC assay are reported to be 3.1 and 1.9, respectively (**Appendix D**). If metabolic activation is included in the experimental protocol, then a positive control requiring metabolic activation will need to be included in each experiment to demonstrate the adequacy of the exposure conditions. An appropriate positive control for such studies has not yet been identified.

12.2.6 Within-Test Replicates

The IC_{50} value of the reference compound (i.e., 17β-estradiol), the positive control, and each test substance should be based on triplicate measurements at each dose level.

12.2.7 Dose Spacing

Generally, to obtain a binding curve, the concentrations of the reference estrogen and the test substances should be spaced by one order of magnitude (i.e., 1 nM, 10 nM, etc.) over the concentration range of interest (1 nM to 1 mM). This results in testing seven concentrations of the test substance in each test. If the range of doses is reduced, then equivalent spacing (e.g., half-log doses) of the seven doses over the smaller dose range should be used.

12.2.8 Data Analysis

Following the measurement of saturation binding of radiolabeled 17β-estradiol to the ER, and after correcting for nonspecific binding, the binding of 17β-estradiol is plotted against the log of the concentration of radiolabeled 17β-estradiol. The curve is analyzed with nonlinear regression techniques to determine B_{max} and K_d. Although a Scatchard analysis (Scatchard, 1949) is frequently used to obtain the K_d, this method has many disadvantages and is not recommended as the primary method (see **Section 2**). Competitive binding experiments use a constant concentration of radiolabeled 17β-estradiol to measure its displacement from the ER by varying concentrations of reference estrogen or test substance. These data are analyzed by nonlinear regression analysis to determine the IC_{50} of the test substance or the reference estrogen. The RBA value for the test substance is calculated by dividing the IC_{50} for 17β-estradiol (or other reference estrogen) by the IC_{50} of the test substance and multiplying the result by 100. The K_i is calculated using the Cheng-Prusoff equation (Cheng and Prusoff, 1973) as a means of assessing the reproducibility of the data from experiment to experiment.

$$K_i = \frac{IC_{50}}{1 + \frac{[\text{Radiolabeled } 17\beta\text{ - estradiol}]}{K_d}}$$

12.2.9 Assay Acceptance Criteria

An assay will be considered acceptable for evaluation if the following conditions are met:

- The unlabeled 17β-estradiol standard curve demonstrates that increasing concentrations of unlabeled 17β-estradiol can displace ^{3}H-17β-estradiol, and that the IC_{50} value for 17β-estradiol is approximately equal to the molar concentration of ^{3}H-17β-estradiol plus the K_d (determined by nonlinear regression and viewed by a Scatchard plot);
- The K_d and IC_{50} values for the unlabeled 17β-estradiol standard curve are within the confidence limits for historical data;
- The ratio of total binding in the absence of competitor to the amount of ^{3}H-17β-estradiol added per assay tube is not greater than 10%;
- The K_i, IC_{50}, and RBA values for the concurrent positive control are within the confidence limits for historical data; and
- The solvent control, at the concentration used, did not alter the sensitivity or reliability of the assay.

12.2.10 Evaluation and Interpretation of Results

A substance is classified as positive for binding to the ER if an IC_{50} value can be obtained and an RBA can be calculated. If an IC_{50} cannot be obtained after testing to the limit dose or the highest dose possible, the test substance is usually classified as being "negative" for *in vitro* ER binding. However, due to solubility constraints (for example), some test substances might induce a significant reduction in binding without achieving at least a 50% reduction in the binding of the reference estrogen to the ER. Until additional information becomes available about the significance of this category of dose response curves, such responses should be noted and the substances classified appropriately (e.g., "equivocal") for the test.

12.2.11 Test Report

At a minimum, the test report must include the following information:

Test substance:

- Name, chemical structure, and CASRN, if known;
- Physical nature (solid or liquid), and purity, if known; and
- Physicochemical properties relevant to the study (e.g., solubility, stability, volatility).

Solvent:

- Justification for choice of solvent if other than water or ethanol; and
- Information to demonstrate that the solvent, if other than an established solvent, does not bind to, or otherwise affect, the ER.

Estrogen receptor:

- Type and source of ER (if from a commercial source, the supplier must be identified);
- Isolation procedure or method for making construct if isolated protein used;
- Protein concentration of ER preparation; and
- Method for storage of ER, if applicable.

Test conditions:

- K_d of the reference estrogen;
- Rationale for the concentration of the reference estrogen;
- Composition of buffer(s) used;
- Concentration range of test substance, with justification;
- Volume of vehicle used to dissolve the test substance and the volume of test substance added;
- Incubation time and temperature;
- Type and composition of metabolic activation system, if added;
- Concentration range of positive and solvent/vehicle controls;
- Method used to separate free reference estrogen, if applicable;
- Method for analyzing bound reference substance;
- Methods used to determine K_i and IC_{50} values; and
- Statistical methods used, if any.

Results:

- Extent of precipitation of test substance;
- The solvent control response compared to the negative control;
- IC data for each replicate at each dose level for all substances, including confidence levels or other measure of intra-dose repeatability;

- Calculated K_i and IC_{50} values and confidence limits for 17β-estradiol, the positive control, and the test substance; and
- Calculated RBA values for the positive control and the test substance.

Discussion of the results:

- Historical K_i and IC_{50} values for the reference estrogen, including ranges, means, and standard deviations;
- Reproducibility of the K_i and IC_{50} values of the reference estrogen, compared to historical data;
- Historical solvent and positive control data with ranges, means, and standard deviations;
- Reproducibility of the K_i and IC_{50}/RBA values for the positive control substance, compared to historical data; and
- The nature of the binding dose response relationship for the test substance.

Conclusion:

- Classification of test substance with regard to *in vitro* ER binding activity.

12.2.12 Replicate Studies

Generally, replicate studies are not mandated for screening assays. However, in situations where questionable data are obtained (i.e., the IC_{50} value is not well defined, "equivocal" results are obtained), additional testing using a more narrow range of test substance concentrations to clarify the results of the primary test would be prudent.

12.3 Standardization of ER Binding Assays for Validation

Appendix B provides *in vitro* ER binding assay protocols (or standard operating procedures) provided by five investigators and one protocol for use with a commercially available ER binding test kit (Pan Vera Corporation, Madison, WI, USA). The assay protocols (as titled by the investigator) included in **Appendix B** are:

- The Estrogen Receptor Competitive Binding Assay Using Rat Uterine Cytosol, as provided by Dr. Susan Laws, U.S. EPA, NHEERL, Research Triangle Park, NC, and Mr. Gary Timm, U.S. EPA, Washington, DC.

- The Competitive ER Binding MCF-7 Whole Cell Assay, as provided by Dr. Guy Leclercq, Clinique et Laboratoire de Cancerologie Mammaire, Centre des Tumeurs de l'Universite Libre de Bruxelles, Brussels, Belgium.
- The Fluorescence Polarization Assay of the Competitive Binding of Ligands to Estrogen-Receptor Complexes, as provided by Dr William Allworth, Department of Chemistry, University of New Orleans, New Orleans, LA.
- The Competitive Ligand Binding Assay, as provided by Dr. Timothy Zacharewski, Dept. of Biochemistry, Michigan State University, Lansing, MI.
- The Rat Estrogen Receptor Equilibrium Exchange Assay, as provided by Dr. Weida Tong, Division of Genetic and Reproductive Toxicology, National Center for Toxicological Research, Jefferson, AR.

Inspection of these protocols provides a perspective on how various assays are conducted by different investigators.

12.3.1 Example Recommended General Protocol for Measuring ER Binding Using the RUC Assay

An example *in vitro* ER binding assay test method protocol is provided in the **Annex** to **Section 12** (designated Annex protocol). This recommended general (as opposed to laboratory-specific) protocol for the RUC assay is based on the RUC assay protocol supplied by the U.S. EPA (**Appendix B-5**) and on information obtained from expert U.S. EPA scientists (Drs. S. Laws, R. Cooper, E. Gray) and professional (Drs. J. Pounds, J. Morris) and technical staff at Battelle Pacific Northwest Laboratories. This general protocol takes into account the minimum procedural standards described in **Section 12.2**. Specific differences between the original U.S. EPA protocol and the version provided in the Annex are described in the following sections. The protocol is included solely to provide guidance to investigators interested in developing comparable laboratory-specific protocols; it has not been used to generate experimental data.

12.3.2 Preparation of Rat Uterine Cytosol (Annex Section 3, Appendix B-4 Section I)

- Animal Use: Consistent with U.S. Government policy, a statement has been added to the Annex protocol that all studies utilizing animals should be approved by the Institutional Animal Care and Use Committee (IACUC) or its equivalent.

12.3.3 Standardization of *In Vitro* ER Binding Assays (Annex Section 4, Appendix B-4 Section II)

- Data Analysis: Since the IC_{50} value is a property of the experiment and the K_i a property of the receptor and the test substance, the Annex protocol recommends that the K_i value be calculated and provided, in addition to the RBA value. Calculation and analysis of the K_i value is not considered in the U.S. EPA RUC protocol.
- Standardization Acceptance Criteria: The Annex protocol includes an assessment against published and historical data of the K_i, as well as the more typical IC_{50} values, for unlabeled 17β-estradiol. The K_i value is not considered in the U.S. EPA RUC protocol.
- Standardization Substances: The U.S. EPA RUC protocol proposes that DES, estrone, and ethinyl estradiol be used as positive and R1881 as negative ER binding substances during efforts to standardize the performance of the RUC assay in the laboratory. After consideration of the data provided in **Appendix D** (see **Table 12-1**), the Annex protocol proposes that tamoxifen and coumestrol be used as positive and β-sitosterol as negative ER binding substances for this purpose. Selection of these two positive ER binding substances is based on a desire to use substances that induce an RBA value between two and three orders of magnitude lower than the reference estrogen to assure the sensitivity of the assay. β-Sitosterol was selected as the negative ER binding substance because of the extent of available data (**Table 12-1**). The Annex protocol includes an assessment, where feasible, against published and historical data of the K_i, as well as IC_{50} and RBA values, for these substances.

12.3.4 *In Vitro* ER Competitive Binding Assay Methodology (Annex Section 5, Appendix B-4 Section III)

- Replicate Assay Tubes: The Annex RUC protocol specifies the use of triplicate (rather than duplicate) assay tubes per concentration tested. The additional assay tube will increase the accuracy of each measured response, and thus the accuracy of the calculated IC_{50} and RBA values.
- Solvent and Positive Controls: The Annex RUC protocol states that when testing substances for their ability to bind to the ER, concurrent negative, solvent and positive controls should be included in each experiment. The U.S EPA RUC protocol includes a solvent control and a

substance without ER binding activity as a negative control substance (tested at a single maximal concentration), but does not include negative or positive controls. A positive control substance is included in the Annex RUC protocol to demonstrate the sensitivity of each experiment and to allow for an assessment of variability in the conduct of the assay across time. While the reference estrogen provides some aspects of a positive control (i.e., it demonstrates the functionality of the assay), it does not allow for an evaluation of the variability in RBA values across experiments. The Annex RUC protocol does not recommend the routine use of a negative control substance.

- Stock Solutions: The Annex RUC protocol specifies that test substances be dissolved in water or in a solvent that is miscible with water. For substances not sufficiently water soluble, absolute ethanol or DMSO are proposed as solvents. Other solvents may be used as long as it can be demonstrated that they do not interact with the test system. The U.S. EPA RUC protocol specifies the use of absolute ethanol only. Choice of solvent should depend on which solvent allows the maximum testable concentration of the test substance.
- Serial Dilutions: In the Annex RUC protocol, it is proposed for the purpose of screening for ED substances that the limit dose be 1 mM. This limit dose (unless precluded by solubility constraints) allows for the detection of an IC_{50} value up to five orders of magnitude below that for 17β-estradiol, the reference estrogen. The U.S EPA RUC protocol specifies an upper limit dose of 0.3 mM.
- Evaluation and Interpretation of Results: In the Annex protocol, criteria for specifying a test substance as positive, negative, or equivocal for binding to the ER are provided. The U.S. EPA RUC protocol provides more limited guidance and does not consider the possibility of "equivocal" responses.
- Test Report: The Annex protocol specifies the information to be included in the Test Report; the U.S. EPA RUC protocol does not. Such guidance ensures that the test reports contain all pertinent information.
- Replicate Studies: The Annex protocol specifies situations for conducting replicate studies (i.e., in situations where questionable data are obtained) to clarify the results of the primary test . The U.S. EPA RUC protocol does not address the issue of replicate studies.

12.4 Recommended List of Substances to be Used for Validation of *In Vitro* ER Binding Assays

Table 12-1 provides a recommended list of substances to be used in the assessment of the reliability and comparative performance of existing or new *in vitro* ER binding assays. A number of factors were considered in developing this list, including the number of times the substance had been tested in the RUC assay, the median RBA value of the substance in the RUC assay, and the extent of concordance of the RUC median RBA value with values obtained for the same substance in other *in vitro* ER binding assays. Because the number of substances tested by multiple laboratories in the RUC assay was insufficient to generate the desired number of substances for consideration, selection of additional substances was based on the availability and concordance of multiple test data among the 13 other *in vitro* ER binding assays considered in this BRD, and the resulting median RBA value across assays. The selected substances were sorted according to their median RBA values. Because the spread of values extended over seven orders of magnitude, ranging from 400 to 0.0001, the substances were sorted into six categories in log decrements: $\geq$10, <10-1; <1-0.1; <0.01-0.1, <0.01-0.001; <0.001. Weakly-binding substances (RBA values <0.001) were difficult to identify because they were not always consistently positive in tests within an assay or between different assays. Also included were substances classified as "negative" for ER binding based on the lack of a positive response in multiple assays when tested at dose levels of at least 1 mM in at least one assay.

Table 12-1 Recommended Substances for Validation of *In Vitro* ER Binding Assays

Classification RBA Range	Substance	CASRN	Median RBA Value	Chemical Class	No. Assays in which Tested[a]	No. Assays with a Positive[a]
	DES	56-53-1	200*	Stilbene	14	14
	4-Hydroxytamoxifen	68047-06-3	175*	Triphenylethylene	13	13
	Estrone	53-16-7	48*	Steroid, phenolic	13	13
	Zearalenone	17924-92-4	44*	Acid lactone	10	10
	Estriol	50-27-1	14*	Steroid, phenolic	12	12
<10 to 1	2',4',6',-Trichloro-4-biphenylol	14962-28-8	3.6**	PCB	4	4
	Tamoxifen	10540-29-1	3.1*	Stilbene	14	14
	Bisphenol C2	14868-03-2	2.6*	Diphenylalkane	3	3
	Coumestrol	479-13-0	1.9*	Benzopyrone	11	11
	Mestranol	72-33-3	1.3*	Steroid, nonphenolic	2	2
<1 to 0.1	Nafoxidine	1845-11-0	0.72**	Triphenylethylene	6	5
	Genistein	446-72-0	0.56*	Flavone	11	11
	Norethynodrel	68-23-5	0.22*	Steroid, nonphenolic	3	3
	4-*tert*-Octylphenol	140-66-9	0.20*	Phenol	9	9
	Phloretin	60-82-2	0.069*	Flavone	3	3
<0.1 to 0.01	Bisphenol A	80-05-7	0.056*	Diphenylalkane	12	12
	Kepone	143-50-0	0.027*	Organochlorine	10	9
	Kaempferol	520-18-3	0.025*	Flavone	3	3
	5α-Dihydrotestosterone	521-18-6	0.014*	Steroid, nonphenolic	9	9

Classification RBA Range	Substance	CASRN	Median RBA Value	Chemical Class	No. Assays in which Tested[a]	No. Assays with a Positive[a]
	o,p'-DDT	789-02-6	0.013*	Organochlorine	12	10
<0.01 to 0.001	Naringenin	480-41-1	0.008*	Flavone	8	6
	4-Androstenedione	63-05-8	0.007**	Steroid, nonphenolic	3	1
	4-Chloro-4'-biphenylol	28034-99-3	0.007*	PCB	2	2
	4-Octylphenol	1806-26-4	0.005*	Phenol	5	4
	Methoxychlor	72-43-5	0.001*	Organochlorine	9	5
<0.001 to 0.0001	4-*tert*-Butylphenol	98-54-4	0.0009*	Phenol	1	1
	Morin	480-16-0	0.0005*	Flavone	1	1
	p,p'-DDT	50-29-3	0.0003*	Organochlorine	6	2
	Progesterone	57-83-0	0.0003*	Steroid, nonphenolic	2	1
	Atrazine	1912-24-9	0.0003*	Aromatic amine	6	1
Negative	Simazine	122-34-9	HTD-2000 µM	Triazine	6	0
	β-Sitosterol	83-46-5	HTD-1000 µM	Steroid, nonphenolic	8	0
	Diethylhexyl phthalate	117-81-7	HTD-5000 µM	Phthalate	1	0

Abbreviations: RUC = Rat uterine cytosol, DES = diethylstilbestrol; PCB = polychlorinated biphenyl; DDT = dichlorodiphenyltrichloroethane; HTD= Highest tested dose

[a]Negative test results at maximum tested concentrations <100 µM were excluded from consideration.

*Median RBA value for positive RUC tests

**Not tested in RUC, median RBA value across all other assays (positive tests only)

Five substances were selected for each RBA category and three for the negative category group. To ensure that each RBA category contained a representative sampling of chemical classes, selection was based on the chemical class to which the substance belongs and whether it was representative of a chemical class used in commerce or found in the environment, and whether it is commercially available. The latter criterion was based on whether the substance could be located in a chemical supply catalogue.

The chemical classes of the substances and the number of substances in each class in **Table 12-1** include nonphenolic steroids (6), organochlorines (4), polychlorinated biphenyls (PCBs), including hydroxylated derivatives (2), flavones (5), phenolic steroids (2), phenols (3), diphenylalkanes (2), stilbenes (2), triphenylethylenes (2), an aromatic amine (1), an acid lactone (1), a benzopyrone (1), a phthalate (1), and a triazine (1).

In March 2001, the U.S. EPA provided a list of 25 substances proposed for testing by Battelle Pacific Northwest (Richland, Washington) in an *in vitro* ER binding RUC assay procedure. In January 2002, EPA provided a modified list of 22 substances. Data generated by the U.S. EPA-sponsored study will be used to validate two QSAR models presently being developed by scientists at the FDA NCTR and by Dr. Mekenyan in Bulgaria. The 22 substances were chosen based on the availability of historical data demonstrating the *in vitro* ER binding affinity, ease of purchase at a purity of >98%, and the lack of extensive health and safety requirements for use (S. Laws, personal communication). Representation of all chemical classes was not a high priority.

The range of binding affinity for the chemicals included those expected to be high affinity binders (nM) to low affinity binders (μM and mM) to non-binders. The substances on the U.S. EPA list (**Table 12-2**) were compared to those recommended here. The U.S. EPA list lacks substances in certain chemical classes, such as PCBs and organochlorines, which have been demonstrated to bind to the ER (**Appendix D**). Since these two chemical classes are ubiquitous in the environment, representative substances were included in the list of substances recommended for validation in this BRD. However, due to possible concern

Table 12-2 List of Substances Being Tested in the *In Vitro* RUC Assay by Battelle

Classification RBA Range	Substances	RUC Median RBA Value	No. Times Tested in RUC Assay	Included in Recommended List in BRD
≥ 10	Meso Hexestrol	300	2	No
	17α-Ethinyl estradiol	173	4	No
	17β-Estradiol	Set at 100	Reference estrogen	Yes
	Estrone	48	4	No
	17α-Estradiol	26.5	2	No
	Coumestrol	1.9	2	Yes
	Tamoxifen citrate	1.62	1	No
≤1 to 0.1	Clomiphene citrate	0.72	1	No
	Norethynodrel	0.22	2	Yes
	Bisphenol B	0.12	2	No
<0.1 to 0.01	Bisphenol A	0.056	5	Yes
	4-Nonylphenol	0.033	10	No
	Kaempferol	0.025	1	Yes
	Daidzein	0.023	1	No
<0.01 to 0.001	4-Cumylphenol	0.005	1	No
<0.001 to 0.0001	Ethyl 4-hydroxybenzoate	0.0006	1	No
	Morin	0.0005	1	Yes
	Progesterone	0.0003	1/3*	Yes
	2-*sec*-Butylphenol	0.0003	1	No
	Phenolphthalin	0.0002	1	No
Negative	Corticosterone	Negative (100 µM)	1	No
	2,4,5-Trichloro-phenoxyacetic acid	Negative (1000 µM)	1	No

*The substance was positive in one of three tests.

about the disposal of the PCB congeners (concentrations in excess of 50 ppm require special disposal procedures), inclusion of this chemical class should be considered further.

Eight substances presently being tested by Battelle were not included in the validation list because of limited published data on their activity in the RUC assay (**Appendix D**). These are tamoxifen citrate, clomiphene citrate, 4-cumylphenol, ethyl-4-hydroxybenzoate, 2-*sec*-butylphenol, phenolphthalin, trichloroacetic acid, bisphenol B, corticosterone, and

2,4,5-trichlorophenoxyacetic acid. Rather than tamoxifen citrate, tamoxifen is recommended (the RBA values are similar) because it has been tested 21 times. 4-Nonylphenol was not selected, as much of the published reports used an undefined nonylphenol or a mixture of nonylphenol isomers. Among the substances with the highest binding affinity (RBA values ≥10), both 17β-ethinyl estradiol and *meso*-hexestrol were considered for inclusion in the proposed list of substances to be used in validation studies, but since DES and 4-hydroxytamoxifen had been tested in a wider range of assays, they were selected. Genistein was selected over daidzein in the 1 to 0.1 RBA value range because it had been tested more frequently. Although morin had only been tested once, it was included in the recommended list since it was considered desirable to have one representative flavone, where possible, in each RBA value range. Corticosterone and 2,4,5-trichlorophenoxyacetic acid, the two substances categorized as negative for ER binding in the Battelle list, were excluded from the recommended list due to limited data (i.e., the highest dose tested for corticosterone in any study was 100 μM; there was only a single study on 2,4,5-trichlorophenoxyacetic acid).

In a validation study, it is important to include substances that cover the range of possible responses without necessarily having the same numbers of substances in each of the artificially defined categories. However, for balance, it would seem that it would be desirable to have equal numbers of substances in each RBA category. When available, the results from the Battelle study might be used to modify the recommended list.

12.5 Conclusions and Recommendations

Currently, there are no published guidelines for conducting *in vitro* ER binding studies, and no formal validation studies to assess the reliability or performance of ER binding assays have been performed. To support the further development and characterization of *in vitro* ER binding assays, minimum procedural standards for such assays and a recommended list of test substances for use in validation studies are provided. The minimum procedural standards and recommended test substances are based on a comparative evaluation of the 14 *in vitro* ER binding assays summarized and evaluated in this BRD. The RUC assay, which has been the most widely used method for identifying substances with ER binding activity, is proposed as the standard against which new tests should be evaluated.

The minimum procedural standards consider methods for determining the K_d of the reference estrogen, methods for test substance preparation, the concentration range of the test substance to evaluate (including the limit dose), the use of solvent and positive controls, the number of replicates to use per test substance concentration, dose spacing, data analysis, assay acceptance criteria, evaluation and interpretation of results, minimal information to include in the test report, and the potential need for replicate studies. These minimum procedural standards are provided to ensure that *in vitro* ER binding studies will be conducted to the same minimal standards.

A suggested general protocol for measuring ER binding using the RUC assay was developed based on a submitted U.S. EPA protocol. Aspects of the RUC assay protocol presented included preparation of rat uterine cytosol, standardization of the assay, the saturation radioligand binding assay, the ER competitive binding assay, considerations for standardizing ER binding assays, ER competitive binding assay methodology, preparation of TEDG assay buffer, preparation of the radiolabeled reference estrogen, preparation of unlabeled reference estrogen, selection of ER concentration and assay volume, preparation of the reference estrogen for the standard curve and nonspecific binding measurements, preparation of test substances, preparation of ER assay tubes, preparation of the HAP slurry, separation of ER-bound radiolabeled 17β-estradiol from free labeled and unlabeled 17β-estradiol, extraction and quantification of the radiolabeled reference estrogen bound to ER, data analysis, and report specifications. It is hoped that such guidance will help investigators in their development of laboratory specific protocols for conducting validation studies on *in vitro* ER binding assays.

A number of factors were considered in developing a list of substances to be used in validation efforts, including the number of times the substance had been tested in the RUC assay, the median RBA value of the substance in the RUC assay, and the extent of concordance of the RUC median RBA value with values obtained for the same substance in other *in vitro* ER binding assays. Because the number of substances tested by multiple laboratories in the RUC assay was insufficient to generate the desired number of substances for consideration, selection of additional substances was based on the availability and concordance of multiple test data among the 13 other *in vitro* ER binding assays considered in this BRD. The selected substances were

sorted according to their median RBA values, over seven orders of magnitude, ranging from 400 to 0.0001. Weakly-binding substances (RBA values <0.001) were difficult to identify because they were not always consistently positive in tests within an assay or using different assays. Also included were substances classified as "negative" for ER binding based on the lack of a positive response in multiple assays when tested at doses of at least 1 mM. Five substances were selected for each RBA category and three for the negative category group. To ensure that each RBA category contained a representative sampling of chemical classes, selection was based on the chemical class to which the substance belongs, whether it was representative of a chemical class used in commerce or found in the environment, and whether the substance is commercially available. The latter criterion was based on whether the substance could be located in a chemical supply catalogue.

The resulting list of 33 substances was compared with the U.S. EPA list of 22 substances to be tested in an RUC assay procedure by Battelle. The U.S. EPA list lacks substances in certain chemical classes, such as PCBs and organochlorines, which have been demonstrated to bind to the ER. Since these two chemical classes are ubiquitous in the environment, representative substances were included in the proposed list of validation substances. Eight of the substances on the U.S. EPA list were not considered because of limited published data on their activity in the RUC assay.

Example Protocol for the *In Vitro* Estrogen Receptor (ER) Competitive Binding Assay Using Rat Uterine Cytosol (RUC)

1.0 Purpose of Assay: This assay is used to determine the relative binding affinities of test substances for the estrogen receptor, which is comprised of the ERα and ERβ subtypes, compared to 17β-estradiol. The primary purpose for this assay is as a screening tool to detect substances with possible estrogenic or anti-estrogenic properties. This example protocol is intended to serve as a guide for producing laboratory specific protocols using this and related assays.

2.0 Terminology:

DES: Diethylstilbestrol

DMSO: Dimethyl sulfoxide

^{3}H-17β-estradiol: 17β-estradiol radiolabeled with tritiated thymidine

HAP: Hydroxylapatite

TEDG buffer: 10 mM Tris, 1.5 mM EDTA, 1 mM dithiothreitol, 10% glycerol, pH 7.4

3.0 Preparation of Rat Uterine Cytosol

Note: All studies utilizing animals should be approved prior to implementation by the Institutional Animal Care and Use Committee (IACUC) or its equivalent.

3.1 TEDG Buffer

Prepare buffer; dithiothreitol is added immediately prior to use.

3.2 Collect Uteri

Collect uteri from female rats ovariectomized seven to ten days prior to being humanely killed. Quickly trim fat and mesentery from the uterus. Weigh and record

the weight of each uterus. Uteri may be used immediately or rapidly frozen in liquid nitrogen, and stored at -80°C for up to three months.

Note: Consistency for all assays should be maintained with respect to the age and strain of the animals used.

3.3 Uterine Cytosol

3.3.1 Weigh trimmed uterus and place in ice-cold TEDG buffer at a ratio of 0.1 g of tissue per 1.0 mL TEDG buffer. Homogenize the tissue using an appropriate homogenizer (5-second bursts).

Note: Cool the homogenizer probe prior to homogenizing each sample by placing the probe in ice-cold TEDG buffer. The homogenization tube should be kept in an ice-cold water bath during the homogenizing process.

3.3.2 Transfer the homogenate to pre-cooled centrifuge tubes and centrifuge for 10 minutes at 2,500 x g at 4°C. The pellet contains the nuclear fraction and the supernatant the ER containing cytosol.

3.3.3 Transfer the supernatant to pre-cooled ultracentrifuge tubes, and centrifuge at 105,000 x g for 60 minutes at 4°C.

3.3.4 Combine the cytosol containing ER supernatants from uteri collected the same day and aliquot for immediate use in ER binding assay or for storage at –80°C.

Note: The cytosol can be stored frozen at –80°C for 1 month prior to use in ER binding assay. Do not thaw and re-freeze the cytosol.

3.3.5 Determine the protein content for each batch of cytosol using an appropriate method.

Note: The dithiothreitol in the buffer is not compatible with the Pierce BCA Protein Assay. Typical protein values are 4 -7 mg/mL.

4.0 Standardization of ER Competitive Binding Assays

Prior to routinely conducting the ER competitive binding assays, the methods should be standardized within each laboratory. This can be accomplished in two steps. First, a series of saturation radioligand binding assays should be conducted to demonstrate ER specificity and saturation. Nonlinear regression analysis of these data (e.g., McPherson, 1985; c1997; Motulsky, 1995) and subsequent Scatchard plots will document ER binding affinity (K_d) and the number of receptors (B_{max}). Second, a series of ER competitive binding assays should be conducted using substances (e.g., 17β-estradiol, DES, estrone) with known affinities for the ER. Comparison of IC_{50} values (e.g., the concentration of a substance that inhibits ^{3}H-17β-estradiol binding by 50%) from these assays with reported values in the literature will assist in documenting that the methods are appropriate for routine use in the laboratory.

4.1 Saturation Radioligand Binding Assay: ER saturation binding experiments measure total, non-specific, and specific binding of increasing concentrations of ^{3}H-17β-estradiol under conditions of equilibrium. A graph of specific ^{3}H-17β-estradiol binding versus radioligand concentration should reach a plateau for maximum specific binding indicative of saturation of the ER with the radioligand. In addition, analysis of the data should document the binding of the ^{3}H-17β-estradiol to a single, high-affinity binding site (e.g., K_d = 0.05 to 0.1 nM and a linear Scatchard plot).

Although several saturation radioligand assays may need to be conducted before an optimal saturation curve, K_d, and B_{max} are achieved, a good starting point is to use enough cytosol to provide 50 to 100 µg protein per assay tube. The concentration for ^{3}H-17β-estradiol should range from 0.03 to 3.0 nM in a total assay volume of 0.5 mL. Non-specific binding should be determined by adding unlabeled 17β-estradiol at 100x the concentration of radiolabeled 17β-estradiol. Analysis of these data should use a non-linear regression analysis (e.g., McPherson, 1985; c1997; Motulsky, 1995) with a final display of the data as a Scatchard plot. Rat uterine cytosol

prepared using this protocol will typically yield a K_d of 0.05 to 0.1 nM and B_{max} of 36 -44 fmol ER/100 µg protein (equivalent to 0.072 to 0.088 nM ER, respectively, when 100 µg protein used in total assay volume of 0.5 mL).

An example of a saturation assay worksheet using increasing concentrations of radioligand is provided in **Table 1**.

Note: For this example, a stock solution of unlabeled 17β-estradiol is prepared in absolute ethanol, with all serial dilutions prepared in assay buffer. All concentrations of ^{3}H-17β-estradiol are prepared in assay buffer.

Table 1 Typical 17β-Estradiol Saturation Assay

Tube Number	^{3}H-17β-Estradiol			Unlabeled 17β-Estradiol			Buffer	Cytosol
	Initial Conc. (nM)	Vol. (µL)	Final Conc. (nM)	Initial Conc. (nM)	Vol. (µL)	Final Conc. (nM)	Vol. (µL)	Vol. (µL)
1	0.3	50	0.03	-			350	100
2	0.3	50	0.03	-			350	100
3	0.3	50	0.03	-			350	100
4	0.6	50	0.06	-			350	100
5	0.6	50	0.06	-			350	100
6	0.6	50	0.06	-			350	100
7	0.8	50	0.08	-			350	100
8	0.8	50	0.08	-			350	100
9	0.8	50	0.08	-			350	100
10	1.0	50	0.1	-			350	100
11	1.0	50	0.1	-			350	100
12	1.0	50	0.1	-			350	100
13	3.0	50	0.3	-			350	100
14	3.0	50	0.3	-			350	100
15	3.0	50	0.3	-			350	100
16	6.0	50	0.6	-			350	100
17	6.0	50	0.6	-			350	100
18	6.0	50	0.6	-			350	100
19	10	50	1	-			350	100
20	10	50	1	-			350	100
21	10	50	1	-			350	100
22	30	50	3	-			350	100

Tube Number	^{3}H-17β-Estradiol			Unlabeled 17β-Estradiol			Buffer	Cytosol
	Initial Conc. (nM)	Vol. (μL)	Final Conc. (nM)	Initial Conc. (nM)	Vol. (μL)	Final Conc. (nM)	Vol. (μL)	Vol. (μL)
23	30	50	3	-			350	100
24	30	50	3	-			350	100
25	0.3	50	0.03	30	50	3	300	100
26	0.3	50	0.03	30	50	3	300	100
27	0.3	50	0.03	30	50	3	300	100
28	0.6	50	0.06	60	50	6	300	100
29	0.6	50	0.06	60	50	6	300	100
30	0.6	50	0.06	60	50	6	300	100
31	0.8	50	0.08	80	50	8	300	100
32	0.8	50	0.08	80	50	8	300	100
33	0.8	50	0.08	80	50	8	300	100
34	1.0	50	0.1	100	50	10	300	100
35	1.0	50	0.1	100	50	10	300	100
36	1.0	50	0.1	100	50	10	300	100
37	3.0	50	0.3	300	50	30	300	100
38	3.0	50	0.3	300	50	30	300	100
39	3.0	50	0.3	300	50	30	300	100
40	6.0	50	0.6	600	50	60	300	100
41	6.0	50	0.6	600	50	60	300	100
42	6.0	50	0.6	600	50	60	300	100
43	10	50	1	1000	50	100	300	100
44	10	50	1	1000	50	100	300	100
45	10	50	1	1000	50	100	300	100
46	30	50	3	3000	50	300	300	100
47	30	50	3	3000	50	300	300	100
48	30	50	3	3000	50	300	300	100
49	0.3	50	0.03	^{3}H-17β-estradiol only, for determining total dpms				
50	0.3	50	0.03	^{3}H-17β-estradiol only, for determining total dpms				
51	0.3	50	0.03	^{3}H-17β-estradiol only, for determining total dpms				
52	0.6	50	0.06	^{3}H-17β-estradiol only, for determining total dpms				
53	0.6	50	0.06	^{3}H-17β-estradiol only, for determining total dpms				
54	0.6	50	0.06	^{3}H-17β-estradiol only, for determining total dpms				
55	0.8	50	0.08	^{3}H-17β-estradiol only, for determining total dpms				
56	0.8	50	0.08	^{3}H-17β-estradiol only, for determining total dpms				
57	0.8	50	0.08	^{3}H-17β-estradiol only, for determining total dpms				
58	1.0	50	0.1	^{3}H-17β-estradiol only, for determining total dpms				
59	1.0	50	0.1	^{3}H-17β-estradiol only, for determining total dpms				

Tube Number	^{3}H-17β-Estradiol			Unlabeled 17β-Estradiol			Buffer	Cytosol
	Initial Conc. (nM)	Vol. (μL)	Final Conc. (nM)	Initial Conc. (nM)	Vol. (μL)	Final Conc. (nM)	Vol. (μL)	Vol. (μL)
60	1.0	50	0.1	^{3}H-17β-estradiol only, for determining total dpms				
61	3.0	50	0.3	^{3}H-17β-estradiol only, for determining total dpms				
62	3.0	50	0.3	^{3}H-17β-estradiol only, for determining total dpms				
63	3.0	50	0.3	^{3}H-17β-estradiol only, for determining total dpms				
64	6.0	50	0.6	^{3}H-17β-estradiol only, for determining total dpms				
65	6.0	50	0.6	^{3}H-17β-estradiol only, for determining total dpms				
66	6.0	50	0.6	^{3}H-17β-estradiol only, for determining total dpms				
67	10	50	1	^{3}H-17β-estradiol only, for determining total dpms				
68	10	50	1	^{3}H-17β-estradiol only, for determining total dpms				
69	10	50	1	^{3}H-17β-estradiol only, for determining total dpms				
70	30	50	3	^{3}H-17β-estradiol only, for determining total dpms				
71	30	50	3	^{3}H-17β-estradiol only, for determining total dpms				
72	30	50	3	^{3}H-17β-estradiol only, for determining total dpms				

Abbreviations: Conc. = concentration; Vol. = volume; dpms = disintegrations per minute

4.2 ER Competitive Binding Assay: An ER competitive binding assay measures the binding of a single concentration of ^{3}H-17β-estradiol in the presence of increasing concentrations of a test substance. The competitive binding curve is plotted as total ^{3}H-17β-estradiol binding versus the concentration (log units) of the competitor. The concentration of the test substance that inhibits 50% of the maximum ^{3}H-17β-estradiol binding is the IC_{50} value. Preliminary experiments should evaluate the effect of the ER concentration of the cytosol, assay volume, and ^{3}H-17β-estradiol concentration on the IC_{50} calculation using unlabeled 17β-estradiol. A good starting point for the ER competitive binding assay is to use enough cytosol to provide 50 to 100 μg protein per assay tube, with 0.5 -1.0 nM ^{3}H-17β-estradiol in a total assay volume of 0.5 mL. Once assay conditions have been optimized, additional ER

competitive binding assays should be conducted to test substances with known affinities for the ER. Such substances include tamoxifen, ethynyl estradiol, coumestrol, and estrone as positive ER binding substances, and R1881 (methyltrienolone) as the negative ER binding substance. Data for the unlabeled 17β-estradiol standard curve and each validation substance should be plotted as the percent ^{3}H-17β-estradiol bound versus the molar concentration (log) of competitor. Estimates of IC_{50} values should be determined using appropriate nonlinear curve fitting software (e.g., McPherson, 1985; c1997; Motulsky, 1995). Since the IC_{50} value is a property of the experiment and the K_i a property of the receptor and the test substance, the K_i value should be provided, as well as the RBA value. The K_i value is calculated using the Cheng -Prusoff equation (Cheng and Prusoff, 1973).

When conducting this assay as a screening test for substances with an ability to bind to the ER, concurrent negative, solvent, and positive controls are included in each experiment. The negative control provides assurance that the solvent does not interact with the test system. The solvent should be tested at the highest concentration that is added with the test substance. A positive control substance (e.g., tamoxifen, coumestrol) is included to demonstrate the sensitivity of each experiment and to allow for an assessment of variability in the conduct of the assay across time. Recommended concentrations of the test substance and positive controls to use are from 1 x 10^{-9} to 1 x 10^{-3} M, in log increments.

4.3 Standardized *In Vitro* ER Competitive Binding Assay Acceptance Criteria

4.3.1 Saturation Assays. In general, when evaluating data from ER saturation assays, the following points should be considered:

- As increasing concentrations of ^{3}H-17β-estradiol were used, did the specific binding curve reach a plateau (e.g., was maximum specific binding reached indicating saturation of ER with ligand)?

- Did the data produce a linear Scatchard plot (e.g., non-linear plots generally indicate a problem with the assay such as ligand depletion [concave plot] or incorrect assessment of non-specific binding [convex plot])?
- Is the K_d within an acceptable range (e.g., 0.05 to 0.1 nM)?

  Note: Literature values for K_d using rat uterine cytosolic preparations have varied from 0.05 to 0.5 nM. The variation in K_d may be a reflection of different laboratories using radiolabeled estradiol with a wide range of specific activity (e.g., ^{3}H-17β-estradiol versus ^{125}I-17β-estradiol). In addition, publications by Salomonsson et al. (1994) and Kuiper et al. (1997, 1998) suggest that a lower Kd may be observed when assay conditions minimize ligand depletion, and that slightly different K_d values exist for ERα and ERβ.
- Are the standard errors for the K_d or B_{max} excessive? If the ratio of either the standard error (SE) of the K_d to the K_d, or the SE of the B_{max} to the B_{max} is much larger than 20%, then the methods for the assay should be re-evaluated.
- Is non-specific binding excessive? The value for non-specific binding should be less than 50% of the total binding.

4.3.2 Competitive Binding Assays. In general, the assay should demonstrate that increasing concentrations of unlabeled 17β-estradiol can compete with a single concentration of ^{3}H-17β-estradiol for binding to the ER. Specific questions to evaluate are as follows:

- As a safeguard against ligand depletion, was the total maximal binding no greater than 10% of the amount of ^{3}H-17β-estradiol added per assay tube?

- Were the K_i and IC_{50} values for unlabeled 17β-estradiol reasonable? The IC_{50} value for unlabeled 17β-estradiol should be approximately equal to the molar concentration of ^{3}H-17β-estradiol used in the assay tube plus the K_d (determined by nonlinear analysis and Scatchard plot of data obtained from saturation radioligand binding assays).
- Were the K_i, IC_{50}, and RBA values for the substance used to validate the performance of the assay reasonable based on published and historical data?
- Was the negative control substance unable to inhibit binding of the radiolabeled 17β-estradiol?

5.0 ER Competitive Binding Assay: Working Protocol

5.1 Preparation of Assay Buffer

Prepare TEDG buffer without dithiothreitol, adjust to pH 7.4 and store at 4°C. Add dithiothreitol immediately prior to use in assay.

5.2 Preparation of ^{3}H-17β-Estradiol

Store at 4 to 5°C in the original container. Obtain the highest specific activity (SA) available from the vendor.

Note: The SA should be adjusted for decay over time.

Dilute the radiolabeled 17β-estradiol with TEDG buffer. Each assay tube should contain 0.5 to 1 nM final concentration of ^{3}H-17β-estradiol.

5.3 Solvent and Positive Controls

When testing substances for their ability to bind to the ER, concurrent negative, solvent, and positive controls should be included in each experiment. The negative control provides assurance that the solvent does not interact with the test system.

The solvent should be tested at the highest concentration that is added with the test substance. A positive control substance is included to demonstrate the sensitivity of each experiment and to allow for an assessment of variability in the conduct of the assay across time. A positive control substance (e.g., tamoxifen, coumestrol) is included to demonstrate the sensitivity of each experiment and to allow for an assessment of variability in the conduct of the assay across time. The suggested concentration range of the test substance and positive control to test is from 1×10^{-9} to 1×10^{-3} M, in log increments.

5.4 Selection of Receptor Concentration and Assay Volume

Receptor concentration of the cytosol and assay volume per assay tube should be adjusted to minimize the likelihood of ligand depletion (e.g., ligand depletion occurs when a high percentage of the ^{3}H-17β-estradiol is bound to ER causing the concentration of the unbound (*free*) ^{3}H-17β-estradiol to significantly differ from the concentration of ^{3}H-17β-estradiol that was originally added to the assay tube [Hulme and Birdshall, 1992]). A general rule is to optimize the assay conditions so that the ratio of the total ^{3}H-17β-estradiol bound in the absence of competitor, to the total ^{3}H-17β-estradiol added to each assay tube, is no more than 10%. Decreasing the amount of cytosolic protein and/or increasing the assay volume will generally lower this ratio. Serial dilutions of the cytosol to obtain 50 to 150 µg protein per assay tube in a total assay volume of 500 µL is a good starting point for determining the optimal ER concentration.

5.5 Preparation of 17β-Estradiol for the Standard Curve and Non-Specific Binding (NSB)

Standard Curve: A standard curve using unlabeled 17β-estradiol should be prepared for each ER competitive binding assay. Final concentrations of unlabeled 17β-

estradiol in the assay tubes should range from 1.0×10^{-7} to 1.0×10^{-11} M. Prepare serial dilutions of 17β-estradiol in absolute ethanol to achieve the final concentrations shown below. Use siliconized glass tubes when preparing the standards. **Table 2** shows recommended concentrations for the unlabeled 17β-estradiol standard curve.

5.6 Preparation of Test Substances

5.6.1 Stock Solutions: Test substances must be dissolved in water or in a solvent that is miscible with water. For substances not sufficiently water soluble, absolute ethanol or DMSO are proposed as solvents. Preference is given to absolute ethanol compared to DMSO since this solvent has been used in most of the studies conducted to date. Other solvents may be used as long as it can be demonstrated that they do not interact with the test system.

Table 2 Example of Preparation Procedure for Unlabeled 17β-Estradiol Standard Curve

Concentrations for Unlabeled 17β-Estradiol Standard Curve		
Standards	**Initial 17β-Estradiol Concentration (Molar)**	***Final 17β-Estradiol Concentration (Molar) in ER Assay Tube**
0	0 (ethanol)	0
NSB	5×10^{-6}	1×10^{-7}
S1	5×10^{-7}	1×10^{-8}
S2	5×10^{-8}	1×10^{-9}
S3	1.67×10^{-8}	3.33×10^{-10}
S4	5×10^{-9}	1×10^{-10}
S5	1.67×10^{-9}	3.33×10^{-11}
S6	5×10^{-10}	1×10^{-11}

**When 10 µL of each standard is added to the ER assay tube, the final concentration will be as indicated when the total volume in the ER assay tube is 500 µL.*

Note: Some test substances will not be soluble at this concentration, so adjustments will need to be made in the final concentration of the

serial dilution tubes depending upon the solubility characteristics of specific substances.

5.6.2 Prepare serial dilutions of each test substance in the appropriate solvent to yield the final concentrations as indicated below.

Note: For the purpose of screening, it is proposed that the upper limit dose be 1 mM and that a concentration range from 1 mM to 1 nM, in ten-fold increments, be used. If the upper limit dose must be reduced due to solubility constraints, then equivalent spacing (e.g., half-log doses) of the seven doses over the smaller dose range should be used. The serial dilutions shown in **Table 3** are based upon the addition of 10 µL of each serial dilution of the test substance in a final assay volume of 500 µL. Other ratios can be used as long as the solvent concentration does not exceed 0.2%.

Table 3 Test Substance Concentrations

Serial Dilutions of Test Substance	Initial Concentration (Molar)	*Final Concentration in ER Assay Tube (Molar)
Concentration 1	5.0×10^{-2}	1.0×10^{-3}
Concentration 2	5.0×10^{-3}	1.0×10^{-4}
Concentration 3	5.0×10^{-4}	1.0×10^{-5}
Concentration 4	5.0×10^{-5}	1.0×10^{-6}
Concentration 5	5.0×10^{-6}	1.0×10^{-7}
Concentration 6	5.0×10^{-7}	1.0×10^{-8}
Concentration 7	5.0×10^{-8}	1.0×10^{-9}

**Final Concentration of test substance in assay tube when 10 µL of Initial Concentration is used in a total volume of 500 µL*

5.7 Preparation of ER Assay Tubes

5.7.1 Label 12 x 75 mm round bottom assay tubes (siliconized glass) in triplicate with codes for the untreated negative control, the solvent control, the NSB, seven dose levels of the positive control substance, and seven dose levels of each test substance.

5.7.2 Place assay tubes in ice bath and add the following to each tube:

Components of ER RUC Binding Assay		
50	µL	Adjust amount of uterine cytosol to provide 50 to 100 µg protein/assay tube
430	µL	TEDG Assay Buffer
10	µL	^{3}H-17β-estradiol to yield final concentration of 0.5 to 1.0 nM
10	µL	Unlabeled 17β-estradiol, negative control, or test substance
500	µL	Total volume in each assay tube

5.7.3 Vortex assay tubes.

Note: Make sure that all components are concentrated at the bottom of tube. If any of the liquid remains on the side of the tube, centrifuge assay tubes for 1 minute at 600 x g (4°C) to concentrate fluid at bottom of tube.

5.7.4 Incubate assay tubes at 4°C for 18 to 20 hours. Assay tubes should be placed on a rotator during the incubation period.

5.8 Preparation of 60% HAP Slurry

5.8.1 Prepare 60% HAP slurry the day before the step to separate the bound and free ^{3}H-17β-estradiol, by adding 10 g HAP to 100 mL TEDG buffer and gently mixing. Cap the container and refrigerate (4°C) the HAP slurry overnight (8 to 10 hours). This amount of HAP will generally yield enough slurry for 70 to 100 assay tubes.

5.8.2 Aspirate the supernatant and resuspend the HAP in fresh TEDG buffer. Allow the HAP to settle and repeat the wash step.

5.8.3 After the last wash, resuspend the HAP to a final volume of 60% HAP and 40% buffer. The HAP slurry should be well suspended and ice-cold when used in the separation procedure.

5.9 Separation of Bound ^{3}H-17β-Estradiol -ER and Free ^{3}H-17β-Estradiol

Note: To minimize dissociation of bound ^{3}H-17β-estradiol from the ER during this process, it is extremely important that the buffers and assay tubes be kept ice-cold and that each step be conducted quickly.

5.9.1 Remove ER assay tubes from rotator and place in an ice-water bath. Using an Eppendorf repeating pipet, quickly add 250 µL of HAP slurry (60% in TEDG buffer, well mixed prior to using) to each assay tube.

5.9.2 Vortex the tubes at 5 minute intervals for a total of 15 minutes. *Note:* This is best accomplished by vortexing an entire rack of tubes at once. It is important to keep the assay tubes cold at this point.

5.9.3 Following the incubation period (step 5.8.2), add 2.0 mL of the TEDG buffer, quickly vortex, and centrifuge at 4°C for 10 minutes at 1000 x g.

5.9.4 After centrifugation, immediately decant the supernatant containing the free ^{3}H-17β-estradiol. The HAP pellet will contain the bound ^{3}H-17β-estradiol -estrogen receptors.

Note: This step can be accomplished quickly by placing the assay tubes in a decanting tube racks. All tubes in the rack can be decanted at once, and the tubes immediately placed back in the ice bath.

5.9.5 Add an additional 2.0 mL ice-cold TEDG buffer and vortex briefly to resuspend the pellet. Work quickly and keep assay tubes cold. Centrifuge again at 4°C for 15 minutes at 1000 x g.

5.9.6 Quickly decant and discard the supernatant. Repeat the wash and centrifugation steps once more.

5.9.7 After the final wash, decant the supernatant. Allow the assay tubes to drain briefly for 1 to 5 minutes.

Note: Watch carefully in case the HAP pellet begins to run down the side of assay tube, which may occur if protein concentration in the cytosol is quite low. At this point, the separation of the free ^{3}H-17β-estradiol and bound ^{3}H-17β-estradiol-ER has been completed. Assay tubes may be left at room temperature.

5.10 Extraction and Quantifying ^{3}H-17β-Estradiol bound to ER

5.10.1 Add 1.5 mL of absolute ethanol to each assay tube. Allow the tubes to sit at room temperature for 15 to 20 minutes, vortexing at 5-minute intervals.

5.10.2 Centrifuge the assay tubes for 10 minutes at 1000 x g and 4°C.

5.10.3 Pipet an aliquot (usually 1.0 to 1.5 mL) or decant the supernatant into 20 mL scintillation vials. Add 10 mL scintillation cocktail, cap and shake vial.

Note: If a 1.0 ml aliquot is used for scintillation counting, the DPMs should be adjusted to account for the total radioactivity in 1.5 ml (i.e., DPMs x 1.5 = Total DPMs bound).

5.10.4 Place vials in scintillation counter for determination of DPMs/vial with quench correction.

5.11 Data Analysis

5.11.1 Terminology

- Total ^{3}H-17β-estradiol: DPMs added to each assay tube (e.g., can be converted to concentration of total ^{3}H-17β-estradiol used in the ER assay).

- Total (Maximum) Binding: DPMs in the 0 standard tubes.
- Nonspecific Binding: DPMs in the NSB standard (i.e., 100 x excess of unlabeled 17β-estradiol).
- Specific Binding: DPMs for each concentration of standard or test substance minus the mean DPM of the NSB tubes.

5.11.2 Data Analysis

i. IC_{50} calculation: Data for the unlabeled 17β-estradiol standard curve and each test substance should be plotted as the percentage of ^{3}H-17β-estradiol bound versus the molar concentration (log) of competitor. Estimates of IC_{50} values should be determined using appropriate nonlinear curve fitting software.

ii. Relative Binding Affinity (RBA) values: The RBA values for each test substance and positive control is calculated by dividing the IC_{50} value for 17β-estradiol by the IC_{50} of the test substance or the positive control and expressing the value as a percent (e.g., RBA for 17β-estradiol =100 %).

iii. K_i calculation: Calculate the K_i value from the IC_{50} value using the Cheng-Prusoff (1973) equation (Cheng and Prusoff, 1973).

5.12 Assay Acceptance Criteria

5.12.1 Unlabeled 17β-estradiol Standard Curve. The assay should demonstrate that increasing concentrations of unlabeled 17β-estradiol can displace ^{3}H-17β-estradiol, and the IC_{50} value for 17β-estradiol should be approximately equal to the molar concentration of ^{3}H-17β-estradiol plus the K_d (determined by nonlinear regression and viewed by a Scatchard plot).

5.12.2 The K_d and IC_{50} values for the unlabeled 17β-estradiol standard curve should be within the confidence limits for historical data.

5.12.3 The ratio of total binding in the absence of competitor to the amount of ^{3}H-17β-estradiol added per assay tube should not be greater than 10%.

5.12.4 The K_i, IC_{50}, and RBA values for the concurrent positive control should be within the confidence limits for historical data.

5.12.5 The solvent control substance, at the concentration used, should not alter the sensitivity or reliability of the assay.

5.13 Evaluation and Interpretation of Results

A substance is classified as positive for binding to the ER if a K_i and IC_{50} values can be obtained and an RBA value can be calculated. If K_i/IC_{50} values cannot be obtained after testing to the upper limit dose or the highest dose possible, the test substance is classified as "negative" for *in vitro* ER binding. However, due to solubility constraints (for example), some test substances might induce a significant reduction in binding but without achieving at least a 50% reduction in the binding of the reference estrogen to the ER. Until additional information becomes available about the significance of this category of dose response curves, such responses should be noted and the substances classified appropriately (e.g., "equivocal") for the test.

5.14 Test Report

The test report must include, but is not limited to, the following information:

5.14.1 Test Substance

- Name, chemical structure, and CASRN, if known;
- Physical nature (solid or liquid), and purity, if known; and
- Physicochemical properties relevant to the study (e.g., solubility, stability, volatility).

5.14.2 Solvent/Vehicle

- Justification for choice of solvent/vehicle if other than water or ethanol;

- Information to demonstrate that the solvent/vehicle, if other than an established solvent, does not bind to, or otherwise affect, the ER.

5.14.3 Estrogen Receptor

- Type and source of ER (if from a commercial source, the supplier must be identified);
- Isolation procedure from tissues, method for making construct, procedure for isolating protein or construction of fusion protein if used;
- Protein concentration of ER preparation; and
- Method for storage of ER, if applicable.

5.14.4 Test Conditions

- K_d of the reference estrogen;
- Rationale for the concentration of the reference estrogen;
- Composition of buffer(s) used;
- Concentration range of test substance, with justification;
- Volume of vehicle used to dissolve test substance and volume of test substance added;
- Incubation time and temperature;
- Type and composition of metabolic activation system, if added;
- Concentration range of positive and solvent/vehicle controls;
- Method used to separate free reference estrogen, if applicable;
- Method for analyzing bound reference substance;
- Methods used to determine K_i and IC_{50} values; and
- Statistical methods used, if any.

5.14.5 Results

- Extent of precipitation of test substance;
- The solvent control response compared to the negative control;

- IC data for each replicate at each dose level for all substances, including confidence levels or other measure of intra-dose repeatability;
- Calculated K_i and IC_{50} values and confidence limits for 17β-estradiol, the positive control, and the test substance; and
- Calculated RBA values for the positive control and the test substance.

5.14.6 Discussion of the Results

- Historical K_i and IC_{50} values for reference ligand, including ranges, means, and standard deviations;
- Reproducibility of the K_i and IC_{50} values of the reference ligand, compared to historical data;
- Historical positive control data with ranges, means, and standard deviations; and
- Reproducibility of the K_i and IC_{50} values for the positive control substance, compared to historical data.

5.14.7 Conclusion

- Classification of test substance with regard to *in vitro* ER-binding activity.

5.15 Replicate Studies

Generally, replicate studies are not mandated for screening assays. However, in situations where questionable data are obtained (i.e., the IC_{50} value is not well defined), replicate tests to clarify the results of the primary test would be prudent.

References

Blair, R.M., Fang, H., Branham, W.S., Hass, B.S., Dial, S.L., Moland, C.L., Tong, W., Shi, L., Perkins, R., Sheehan, D.M. (2000) The estrogen receptor relative binding affinities of 100 natural and xenochemicals: structural diversity of ligands. Toxicol Sci 54:138-153.

Hulme, E.C. and Birdsall, N.J.M. (1992) Strategy and tactics in receptor-binding studies. In: Receptor ligand interactions: a practical approach. Ed., E.C. Hulme. IRL Press, New York. pp. 63-76.

Korach, K.S. and Muldoon, T.G. (1974) Studies on the nature of the hypothalamic estradiol-concentrating mechanism in the male and female rat. Endocrinology 94:785-793.

Kuiper, G., Carlsson, B., Grandien, K., Enmark, E., Haggblad, J., Nilsson, S., Gustafsson, J. (1997) Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology 138(3):863-870.

Kuiper, G., Lemmen, J., Carlsson, B., Corton, J.C., Safe, S., Van Der Saag, P., Van Der Burg, B., Gustafsson, J. (1998) Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. Endocrinology 139(10):4252-4263.

Laws, S.C., Carey, S.A., Hart, D.W., Cooper, R.L. (1994) Lindane does not alter the estrogen receptor or the estrogen-dependent induction of progesterone receptors in sexually immature or ovariectomized adult rats. Toxicology 92:127-142.

Laws, S.C., Carey, S.A., Ferrell, J.M., Bodman, G.J., Cooper, R.L. (2000) Estrogenic activity of octylphenol, nonylphenol, bisphenol A and methoxychlor in rats. Toxicol Sci 54:154-167.

McPherson, G.A. (1985) Analysis of radioligand binding experiments. A collection of computer programs for the IBM PC. J Pharmacol Methods:14:213-228.

McPherson, G.A. (c1997) BioSoft KELL: A collection of programs for the analysis of radioligand binding experiments (KINETIC, EBDA, LIGAND, LOWRY for Windows), Ferguson, MO.; http://www.biosoft.com.

Motulsky, H.J. (1995) Analyzing data with GraphPad Prism. GraphPad Software Inc., San Diego, CA.

Salomonsson, M., Carlsson, B., Haggblad, J. (1994) Equilibrium hormone binding to human estrogen receptors in highly diluted cell extracts is non-cooperative and has a Kd of approximately 10 pM. J Steroid Biochem Mol Biol 50:313-318.

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13.0 REFERENCES

Adami, H., Bergstrom, R., Mohner, M., Zaronski, W., Storm, H., Ekbom, A., Tretli, S., Teppo, L., Ziegler, H., and Rahu, M. (1994) Testicular cancer in nine Northern European countries. Int J Cancer 59:33-38.

Ankley, G., Mihaich, E., Stahl, R., Tillitt, D., Colborn, T., McMaster, S., Miller, R., Bantle, J., Campbell, P., Denslow, N., Dickerson, R., Folmar, L., Fry, M., Giesy, J., Gray, L.E., Guiney, P., Hutchinson, T., Kennedy, S., Kramer, V., LeBlanc, G., Mayes, M., Nimrod, A., Patino, R., Peterson, R., Purdy, R., Ringer, R., Thomas, P., Touart, L., Van der Kraak, G., and Zacharewski, T. (1998) Overview of a workshop on screening methods for detecting potential (anti-) estrogenic/androgenic chemicals in wildlife. Environ Toxicol and Chem 17:68-87.

Barlow, S., Kavlock, R.J., Moore, J.A., Schantz, S.L., Sheehan, D.M., Shuey, D.L., and Lary, J.M. (1999) Teratology Society Public Affairs Committee position paper: developmental toxicity of endocrine disruptors to humans. Teratology 60:365-375.

Baulieu, E.E., and Raynaud, J.P. (1970) A "proportion graph" method for measuring binding systems. Eur J Biochem 13:293-304.

Blair, R.M., Fang, H., Branham, W.S., Hass, B.S., Dial, S.L., Moland, C.L., Tong, W., Shi, L., Perkins, R., and Sheehan, D.M. (2000) The estrogen receptor relative binding affinities of 188 natural and xenochemicals: Structural diversity of ligands. Toxicol Sci 54:138-153.

Bolger, R., Wiese, T.E., Ervin, K., Nestich, S., and Checovich, W. (1998) Rapid screening of environmental chemicals for estrogen receptor binding capacity. Environ Health Perspect 106:551-557.

Bortone, S.A., Davis, W.P., and Bundrick, C.M. (1989) Morphological and behavioral characters in mosquitofish as potential bioindication of exposure to kraft mill effluent. Bull Environ Contam Toxicol 43:370-377.

Brzozowski, A.M., Pike, A.C.W., Dauter, Z., Hubbard, R.E., Bonn, T., Engstrom, O., Ohman, L., Greene, G.L., Gustafsson, J.-Å., and Carlquist, M. (1997) Molecular basis of agonism and antagonism in the oestrogen receptor. Nature 389:753-758.

Carlsen, E., Giwercman, A., Keiding, N., and Skakkabaek, N.E. (1992) Evidence for decreasing quality of semen during the last 50 years. Brit Med J 305:609-613.

Charles, G.D., Bartels, M.J., Gennings, C., Zacharewski, T.R., Freshour, N.L., Gollapudi, B.B., and Carney, E.W. (2000) Incorporation of S-9 activation into an ER-α transactivation assay. Reprod Toxicol 14: 207-216.

Checovich, W.J., Bolger, R.E., and Burke, T. (1995) Fluorescence polarization -- a new tool for cell and molecular biology. Nature 375:254-256.

Cheng, Y., and Prusoff, W.H. (1973) Relationship between the inhibition constant (Ki) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. Biochem Pharmacol 22:3099-108.

Cheskis, B.J., Karathanasis, S., and Lyttle C.R. (1997) Estrogen receptor ligands modulate its interaction with DNA. J Biol Chem 272:11384-11391.

Clark, J.H., and Gorski, J. (1969) Estrogen-receptors: An evaluation of cytoplasmic-nuclear interactions in a cell-free system, and a method for assay. Biochim Biophys Acta 192:508-515.

Combes, R.D. (2000) Endocrine disruptors: A critical review of *in vitro* testing strategies for assessing their toxic hazard to humans. ATLA 28:81-118.

Dandliker, W.B., Hsu, M.L., Levin, J., and Rao, B.R. (1981) Equilibrium and kinetic inhibition assays based upon fluorescence polarization. Methods Enzymol 74:3-28.

Erdos, T., Best-Belpomme, M., and Bessada, R. (1970) A rapid assay for binding estradiol to uterine receptor(s). Anal Biochem 37:244-252.

Fang, H., Tong, W., Shi, L.M., Blair, R., Perkins, R., Branham, W., Hass, B.S., Xie, Q., Dial, S.L., Moland, C.L., and Sheehan, D.M. (2001) Structure-activity relationships for a large diverse set of natural, synthetic, and environmental estrogens. Chem Res Toxicol 14:280-294.

FDA. (2002) Good laboratory practices for nonclinical laboratory studies. 21 CFR 58.

Federal Food, Drug, and Cosmetic Act (FFDCA) of 1996. 21 CFR 9.

Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) of 1947 (7 U.S.C. 1947) and FIFRA amendments (1972, 1975, 1978, 1980).

Federal Register (Vol. 66, No. 57, pp. 16278 – 16279. March 23, 2001). Available: http://iccvam.niehs.nih.gov/methods/endocrine.htm [accessed 15 July 2002].

Federal Register (Vol. 67, No. 66, pp. 16415-16416. April 5, 2002). Available: http://iccvam.niehs.nih.gov/methods/endocrine.htm [accessed 15 July 2002].

Folmar, L.C., Denslow, N.D., Rao, V., Chow, M., Crain, D.A., Enblom, J., Marcino, J., and Guillette, L.J., Jr. (1996) Vitellogenin induction and reduced serum testosterone concentrations in feral male carp (*Cyprinus carpio*) captured near a major metropolitan sewage treatment plant. Environ Health Perspect 104:1096-1101.

Gaido, K.W., Leonard, L.S., Maness, S.C., Hall, J.M., McDonnell, D.P., Saville, B., and Safe, S.H. (1999) Differential interaction of the methoxychlor metabolite 2,2-Bis-(*p*-hydroxyphenyl)-1,1,1-trichloroethane with estrogen receptors α and β. Endocrinology 140:5746-5753.

Glass, A.G., and Hoover, R.N. (1990) Rising incidence of breast cancer relationship to age and receptor status. J Natl Cancer Inst. 82:693-696.

Gorski, J., Toft, D., Shyamala, G., Smith, D., and Notides, A. (1968) Hormone receptors: Studies on the interaction of estrogen with the uterus. Recent Progr Hormone Res 24:45-72.

Gray, L.E., Jr., Kelce, W.R., Wiese, T., Tyl, R., Gaido, K., Cook, J., Klinefelter, G., Desaulniers, D., Wilson, E., Zacharewski, T., Waller, C., Foster, P., Laskey, J., Reel, J., Giesy, J., Laws, S., McLachlan, J., Breslin, W., Cooper, R., DiGiulio, R., Johnson, R., Purdy, R., Mihaich, E., Safe, S., Sonnenschein, C., Welshons, W., Miller, R., McMaster, S., and Colborn, T. (1997) Endocrine screening methods workshop report: Detection of estrogenic and androgenic hormonal and antihormonal activity for chemicals that act via receptor or steroidogenic enzyme mechanisms. Reprod Toxicol 11: 719-750.

Hähnel, R. (1971) Properties of the estrogen receptor in the soluble fraction of human uterus. Steroids 17:105-132.

Hulme, E.C., and Birdsall, N.J.M. (1992) Chapter 4: Strategy and tactics in receptor-binding studies. Receptor –Ligand Interactions: A Practical Approach. Oxford University Press, NY. ed. Hulme, E.C. pp. 63 – 176.

Hwang, K.J., Carlson, K.E., Anstead, G.M., and Katzenellenbogen, J.A. (1992) Donor-accepted tetrahydrochrysenes, inherently fluorescent, high-affinity ligands for the estrogen receptor: binding and fluorescence characteristics and fluorometric assay of receptor. Biochemistry 31:11536-11545.

ICCVAM. (1999) Evaluation of the Validation Status of Toxicological Methods: General Guidelines for Submissions to ICCVAM. NIH Publication No. 99-9946. National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.
Available: http://iccvam.niehs.nih.gov/docs/guidelines/subguide.pdf [accessed 15 July 2002].

ICCVAM. (1997) Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the *ad hoc* Interagency Coordinating Committee on the Validation of Alternative Methods. NIH Publication No. 97-3981. National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.
Available: http://iccvam.niehs.nih.gov/docs/guidelines/validate.pdf [accessed 15 July 2002].

Jameson, D.M., and Sawyer, W.H. (1995) Fluorescence anisotropy applied to biomolecular interactions. Methods Enzymol 246:283-300.

Jensen, E.V., Suzuki, T., Kawashima, T., Stumpf, W.E., Jungblut, P.W., and DeSombre, E.R. (1968) A two-step mechanism for the interaction of estradiol with rat uterus. Proc Natl Acad Sci USA 59:632-638.

Jungblut, P.W., Hughes, S., Hughes, A., and Wagner, R.K. (1972) Evaluation of various methods for the assay of cytoplasmic oestrogen receptors in extracts of calf uteri and human breast cancers. Acta Endocrinol 70:185-195.

Kassis J.A., Walent J.H., and Gorski J. (1986) Estrogen receptors in cultured rat uterine cells: induction of progesterone receptors in the absence of estrogen receptor processing. Endocrinology 118:603-608.

Kavlock, R.J., Daston, G.P., DeRosa, C., Fenner-Crisp, P., Gray, L.E., Kaatari, S., Lucier, G., Luster, M., Mac, M.J., Maczka, C., Miller, R., Moore, J., Rolland, R., Scott, G., Sheehan, D.M., Sinks, T., and Tilson, H. (1996) Research needs for the risk assessment of health and environmental effects of endocrine disruptors: A report of the U.S. EPA-sponsored workshop. Environ Health Perspect 104(Suppl 4):715-740.

Korenman, S.G. (1970) Relation between estrogen inhibitory activity and binding to cytosol of rabbit and human uterus. Endocrinology 87:1119-1123.

Kuiper, G.G.J.M., Carlsson, B., Grandien, K., Enmark, E., Haggblad, J., Nillson, S., and Gustafsson, J.-Å. (1997) Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology 138:863-870.

Kuiper G.G.J.M., and Gustafsson J.-Å. (1997) The novel estrogen receptor-beta subtype: potential role in the cell- and promoter-specifications of estrogens and anti-estrogens. FEBS Lett 410:87-90.

Kuiper, G.G.J.M., Lemmen, J.G., Carlsson, B., Corton, J.C., Safe, S.H., van der Saag, P.T., van der Burg, B., Gustafsson, J.-Å. (1998) Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. Endocrinology 139:4252-4263.

Kumar, V., Green, S., Stack, G., Berry, M., Jin, J.-R., and Chambon, P. (1987) Functional domains of the human estrogen receptor. Cell 51:941-951.

Kupfer, D. (1987-88) Critical evaluation of methods for detection and assessment of estrogenic compounds in mammals: Strengths and limitations for application to risk assessment. Reprod Toxicol 1: 147-153.

Love, J.D., Gooch, J.T., Nagy, L., Chatterjee, V.K.K., and Schwabe, J.W.R. (2000) Transcriptional repression by nuclear receptors: Mechanisms and role in disease. Biochem Soc Trans 28:390-396.

Lundblad, J.R, Laurance, M, and Goodman, R.H. (1996) Fluorescence polarization analysis of protein-DNA and protein-protein interactions. Mol Endocrinol 10:607-612.

Matthews, J., Celius, T., Halgren, R., and Zacharewski, T. (2000) Differential estrogen receptor binding of estrogenic substances: A species comparison. J Steroid Biochem Mol Biol 74:223-234.

Matthews, J., and Zacharewski, T. (2000) Differential binding affinities of PCBs, HO-PCBs, and aroclors with recombinant human, rainbow trout (*Onchorhynkis mykiss*), and green anole (*Anolis carolinensis*) estrogen receptors, using a semi-high throughput competitive binding assay. Toxicol Sci 53:326-339.

McDonnell, D.P. (1999) The molecular pharmacology of SERMs. Trends Endocrinol Metab 10:301-311.

The Merck Index: Twelfth Edition on CD-ROM. (1996). Whitehouse Station, NJ: Merck & Co, Inc.

Mekenyan, O.G., Kamenska V., Schmieder P.K., Ankley G.T., Bradbury S.P. (2000). A computationally based identification algorithm for estrogen receptor ligands: Part 2. Evaluation of a hERα binding affinity model. Toxicol Sci 58:270-281

Motulsky, H. The GraphPad Guide to Analyzing Radioligand Binding Data. ©1995-1996. GraphPad Software, Inc, San Diego, CA. Available: http://www.graphpad.com [Accessed on 12 July 2002].

Muller, J., Rajpert-De Meyts, E., Scheike, T., Sharpe, R., Sumpter, J., and Skakkebaek, N.E. (1996) Male reproductive health and environmental xenoestrogens. Environ Health Perspect 104(Suppl 4):741-803.

NAS. (1999) Hormonally Active Agents in the Environment. National Academy Press, Washington, DC.

National Library of Medicine, Specialized Information Services, Chemical Information. ChemIDPlus. Available: http://sis.nlm.nih.gov/Chem/ChemMain.html [Accessed on 12 July 2002].

Noteboom, W., and Gorski, J. (1965) Stereospecific binding of estrogens in the rat uterus. Arch Biochem Biophys 111:559-568.

Notides, A.C. (1970) Binding affinity and specificity of the estrogen receptor of the rat uterus and anterior pituitary. Endocrinology 87:987-992.

Ozers, M.S., Hill, J.J., Ervin, K., Wood, J.R., Nardulli, A.M., Royer, C.A., and Gorski, J. (1997) Equilibrium binding of estrogen receptor with DNA using fluorescence anisotropy. J Biol Chem 272(48):30405-30411.

Petit, F., Valotaire, Y., and Pakdel, F. (1995) Differential functional activities of rainbow trout and human estrogen receptors expressed in the yeast *Saccharomyces cerevisiae*. Eur J Biochem 233:584-92.

Phillips, B. (2000) Comments on the European Community strategy for endocrine disruptors. ATLA 28:517-522.

Pike, A.C.W., Brzozowski, A.M., Walton, J., Hubbard, R.E., Bonn, T., Gustafsson, J.-Å., and Carlquist, M. (2000) Structural aspects of agonism and antagonism in the oestrogen receptor. Biochem Soc Trans 28(part 4):396-400.

Public Law 104-170. Food Quality Protection Act (FQPA) of 1996. Available: http://www.epa.gov/opppsps1/fqpa/gpogate.pdf [accessed 15 July 2002].

Public Law 104-182. Safe Drinking Water Act Amendments (SDWA) of 1996. Available: http://www.epa.gov/safewater/sdwa/theme.html [accessed 15 July 2002].

Public Law 105-115. Food and Drug Administration Modernization Act of 1997. Available: http://www.access.gpo.gov/nara/publaw/105publ.html [accessed 26 July 2002].

Public Law 106-545. ICCVAM Authorization Act of 2000. [114 Stat. 2721]. Available: http://iccvam.niehs.nih.gov/about/PL106545.pdf [accessed 15 July 2002].

Puca, G.A., and Bresciani, F. (1969) Association constant and specificity of oestradiol-receptor interaction. Nature 223:745-747.

Purdom, C.E., Hardiman, P.A., Bye, V.J., Eno, N.C., Tyler, C.R., and Sumpter, J.P. (1994). Estrogenic effects of effluents from sewage treatment works. Chem Ecol 8:275-285.

Safe S.H. (2000) Endocrine disruptors and human health--is there a problem? An update. Environ Health Perspect 108:487-493

Salomonsson, M., Carlsson B., Haggblad, J. (1994). Equilibrium hormone binding to human estrogen receptors in highly diluted cell extracts is non-cooperative and has a Kd of approximately 10 pM. J Steroid Biochem Molec Biol 50(5/6):313-318.

Scatchard, G. (1949) The attractions of proteins for small molecules and ions. Ann NY Acad Sci 51:660-672.

Shafie, S., and Brooks, S.C. (1979) Characteristics of the dextran-coated charcoal assay for estradiol receptor in breast cancer preparations. J Lab Clin Med 94:784-798.

Sharpe, R.M., and Skakkabaek, N.E. (1993) Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? Lancet 341:1992.

Shelby, M.D., Newbold, R.R., Tully, D.B., Chae, K., and Davis, V.L. (1996) Assessing environmental chemicals for estrogenicity using a combination of *in vitro* and *in vivo* assays. Environ Health Perspect 104:1296-1300.

Shyamala, G, and Gorski, J. (1968) Estrogen receptors in the rat uterus. Studies on the interaction of cytosol and nuclear binding sites. J Biol Chem 244:1097-1103.

Sumida, K., Ooe, N., Nagahori, H., Saito, K., Isobe, N., Kaneko, H., and Nakatsuka, I. (2001) An *in vitro* reporter gene assay method incorporating metabolic activation with human and rat S9 or liver microsomes. Biochem Biophys Res Commun 280: 85-91.

Toft, D., and Gorski, J. (1965) A receptor protein for estrogens: its isolation from the rat uterus and preliminary characterization. J Cell Biol 27:107A-108A.

Toft, D., and Gorski, J. (1966) A receptor molecule for estrogens: isolation from the rat uterus and preliminary characterization. Proc Natl Acad Sci USA 55:1574-1581.

Toft, D., Shyamala, G., and Gorski, J. (1967) A receptor molecule for estrogens: Studies using a cell-free system. Proc Natl Acad Sci USA 57:1740-1743.

Toppari, J., Larsen, J.C., Christiansen, P., Giwercman, A., Grandjean, P., Guillette, L.J. Jr., Jegou, B., Jensen, T.K., Jouannet, P., Keiding, N., Leffers, H., McLachlan, J.A., Meyer, O., Muller, J., Rajpert-De Meyts, E., Scheike, T., Sharpe, R., Sumpter, J., and Skakkebaek, N.E. (1996) Male reproductive health and environmental xenoestrogens. Environ Health Perspect 104(Suppl 4):741-803.

Toxic Substances Control Act (TSCA) of 1976. 15 U.S.C. Sections 2601-2629. Available: http://www.epa.gov/region5/defs/html/tsca.htm [accessed 15 July 2002].

U.S. EPA. (1997) Special Report on Environmental Endocrine Disruption; An Effects Assessment and Analysis. EPA/630/R-96/012. Available: http://www.epa.gov/ORD/WebPubs/endocrine/endocrine.pdf [accessed 15 July 2002].

U.S. EPA. (1998a) Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) Final Report. EPA/743/R-98/003. Available: http://www.epa.gov/scipoly/oscpendo/history/finalrpt.htm [accessed 15 July 2002].

U.S. EPA. (1998b) Endocrine Disruptor Screening Program; Proposed Statement of Policy. 63 *FR* 71542-71568.

U.S. EPA. (1999) Review of the EPA's Proposed Environmental Endocrine Disruptor Screening Program by a Joint Subcommittee of the Science Advisory Board and Scientific Advisory Panel. EPA-SAB-EC-99-013.

U.S. EPA. (2000) Environmental Protection Agency: Report to Congress – August 2000.

U.S. EPA. (2001) Good Laboratory Practices. Toxic Substances Control Act. 40 CFR 792.

U.S. EPA. (2002) Good Laboratory Practice Standards. Federal Insecticide, Fungicide and Rodenticide Act. 40 CFR 160.

Zacharewski, T.R. (1998) Identification and assessment of endocrine disruptors: Limitations of *in vivo* and *in vitro* assays. Environ Health Perspect 106(Suppl 2): 577-582.

Zacharewski, T.R. (1997) *In vitro* bioassays for assessing estrogenic substances. Environ Sci Technol 31: 613-623.

14.0 GLOSSARY[1]

Accuracy[2]: A measure of test performance. (a) The closeness of agreement between a test result and an accepted reference value; (b) The proportion of correct outcomes of a method. Often used interchangeably with **concordance**.

Activation (of genes): The interaction of specific molecules or molecular complexes with specific genes to initiate their expression (transcription of mRNA).

Affinity (high; low): The strength of binding of a molecule to a receptor protein.

Agonism: The binding of a substance to a receptor to initiate effects similar to those produced by the natural ligand for the receptor.

Agonist: A substance that mimics the action of an endogenous hormone.

Androgen: A class of steroid hormone, which includes testosterone and 5α-dihydrotestosterone, responsible for the development and maintenance of the male reproductive system.

Antagonism: The binding of a substance to a receptor to inhibit or counteract the effects produced by the natural ligand for the receptor.

Antagonist: A substance that blocks or diminishes the activity of an **agonist**.

Cell-free: Not containing intact cells. May contain cell or tissue homogenates or artificial mixtures of cellular components.

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[1] The definitions in this Glossary are restricted to their uses with respect to endocrine mechanisms and actions.

[2] Definition used by the Interagency Coordinating Committee on the Validation of Alternative Methods.

Complex mixture: A mixture containing many, generally uncounted, substances, many of which are undefined (e.g., plant homogenates; fuels).

Concordance[2]: A measure of test performance. The proportion of all chemicals that are correctly classified as positive or negative. Often used interchangeably with **accuracy**. The concordance is highly dependent on the **prevalence** of positives in the population being examined.

***C*-Terminal region:** The end of a protein molecule that contains a free carboxylic acid moiety.

Cytoplasm: The material inside the cell, excluding the nucleus, that contains the intracellular fluid, organelles, soluble enzymes, membrane components and other factors.

Cytosol: see Cytoplasm

Detoxification: Reduction of the toxicity (of a substance) by metabolism to a less toxic form, or by removal of the substance from the affected cell or organism.

Dextran: A viscous or semi-viscous polymer of glucose.

Dissociation constant: A measure of the ability of a molecule to be released from binding to a receptor.

DNA-regulatory activity: Refers to a DNA-binding molecule or complex that causes a change in DNA-related activities.

Domain: A region of a protein defined by its activity.

Endocrine disruption: Activity by an exogenous chemical substance that alters the structure or function(s) of the endocrine system and causes adverse effects at the level of the organism, its progeny, populations, or subpopulations of organisms.

Endocrine disruptor: A substance determined to cause endocrine disruption.

Endocrine system: Made up of glands located throughout the body, the hormones that are synthesized and secreted by the glands into the bloodstream, and the receptors in the various tissues are organs that recognize and respond to the hormones.

Endogenous: Originating within the organism of interest.

Endpoint: The biological process, response, or effect assessed by a test method.

Estrogen: A class of steroid hormones, which includes 17β-estradiol, responsible for regulation of specific female reproductive functions and for development and maintenance of the female reproductive system.

Estrogenic: Having biological activity similar to that of an estrogen.

Exogenous: Originating outside the organism of interest.

False negative[2]: An active substance incorrectly identified as negative by a test.

False negative rate[2]: The proportion of all positive (active) substances falsely identified as negative. A measure of test performance.

False positive[2]: An inactive substance incorrectly identified as positive by a test.

False positive rate[2]: The proportion of all negative (inactive) substances falsely identified as positive. A measure of test performance.

Fluorescence polarization (FP): A technique that can detect molecular interactions by monitoring changes in the polarization of fluorescently labeled or inherently fluorescent molecules.

Frog metamorphosis assay: A test method that measures the ability of a substance to affect the metamorphosis of frog larvae (tadpoles) to adults.

Gonadal recrudescence assay: A test method that measures the ability of a substance to produce effects in estrogen- and androgen-dependent accessory sex organs or gonad maturation in fish. A test method for potential estrogen- and androgen-related endocrine disruption.

Half-life: The time it takes for a chemical or radioactive substance to lose half its activity.

Hazard: An adverse health or ecological effect.

Hershberger assay: Measures the ability of a substance to alter the weight of androgen-dependent accessory sex organs (e.g., ventral prostate or seminal vesicles) or tissues in castrated rats or mice. A test method for potential androgen and anti-androgen related endocrine disruption activity.

Homology (DNA): Similarity in DNA sequence of segments or genes from different strains or species of organisms.

Hormone: A chemical substance produced in specific cells, or glands, that can either act locally or be released into the bloodstream to act on an organ or tissue in another part of the body.

Hydrophobic: Refers to chemicals and substances that will not dissolve or that sparingly dissolve in water.

Hydroxyapatite (HAP): A form of calcium phosphate with the ability to bind to some classes of organic molecules.

Hypospadias: A clinical condition in newborns that manifests itself as a displaced opening of the urethra. Occurs in males only and is considered a fetal developmental anomaly.

Interlaboratory reproducibility[2]: A measure of whether different laboratories using the same protocol and test chemicals can produce qualitatively and quantitatively similar results. See **reliability**.

Intralaboratory reproducibility[2]: A measure of whether the same laboratory can successfully replicate results using a specific test protocol at different times. See **reliability**.

Intraperitoneal: Administration by injection directly into the peritoneal cavity.

In vitro: In glass. Refers to assays that are carried out in an artificial system (e.g., in a test tube or petri dish) and typically use single-cell organisms, cultured cells, cell-free extracts, or purified cellular components.

In vivo: In the living organism. Refers to assays performed in multicellular organisms.

K_d: Equilibrium dissociation constant of a reference compound in a specific receptor preparation. A measure of the strength of binding between a receptor and ligand.

K_i: Equilibrium dissociation constant of an inhibitor in a competitive receptor binding experiment.

Ligand: A substance that is capable of binding to a specific receptor protein.

Ligand-binding domain: The area within a receptor molecule that attracts and holds a ligand.

Metabolic activation: Metabolism of a chemical by an organism or a cell-free extract to a biologically active form.

Negative control: An untreated sample containing all reagents of a test system, except the assay solvent, which is replaced with a known non-reactive material, such as water. This sample is processed with treated samples and other control samples to determine whether the solvent interacts with the test system.

Negative predictivity[2]: The proportion of correct negative responses among substances testing negative.

***N*-Terminal region:** The end of a protein molecule that contains a free amino acid moiety.

Ovariectomized: Having the ovaries surgically removed.

Peer review: Objective review of data, a document, or proposal, and provision of recommendations, by an expert individual or group of individuals having no conflict of interest with the outcome of the review.

pH: A measure of the acidity or alkalinity of a solution. pH 7.0 is neutral; higher pHs are alkaline, lower pHs are acidic.

Placental aromatase assay: Measures the ability of a substance to induce or inhibit the activity of the aromatase enzyme, which converts testosterone to estradiol. A test method for potential anti-estrogen related endocrine activity.

Positive control: A sample containing all components of a test system and treated with a substance known to induce a positive response, that is processed with other samples to demonstrate the sensitivity of each experiment and to allow for an assessment of variability in the conduct of the assay over time.

Positive predictivity[2]: The proportion of correct positive responses among substances testing positive.

Prevalence[2]: The proportion of positives in the population of substances tested.

Priority setting: The collection, evaluation, and analysis of existing relevant information to determine whether, and in what relative order of priority, substances will be subjected to screening or testing.

Protocol[2]: The precise, step-by-step description of a test, including the listing of all necessary reagents, criteria and procedures for the evaluation of the test data.

Pubertal female assay: Measures the ability of a substance to induce or inhibit the onset of puberty in immature female rats and mice, measured as an early or late opening of the vagina. A test method for potential estrogenicity and anti-estrogenicity.

Pubertal male assay: Measures the ability of a substance to induce or inhibit prepubertal separation in immature male rats and mice. At recovery (53 days), various tissues are weighed and the thyroid examined histologically. A test method for potential androgen- and anti-androgen related endocrine disruption.

Radiolabel: A radioactive isotope of an atom that is added to a molecule to allow the molecule to be identified by **scintillation counting**.

Receptor: A protein or protein complex, which binds to specific molecules for the purpose of transporting them elsewhere in the cell, or for producing a chemical signal.

Receptor binding assay (competitive): An assay to measure the ability of a substance to bind to a hormone receptor protein, which is typically performed by measuring the ability of the substance to displace the bound natural hormone.

Receptor superfamily: A family of related receptors with similar composition and reactivity (e.g., the estrogen, androgen, and glucocorticoid receptors).

Relevance (of an assay)[2]: The relationship of a test to the effect of interest and whether a test is meaningful and useful for a particular purpose. The extent to which an assay will correctly predict or measure the biological effect of interest. A measure of assay **performance**.

Reliability (of an assay)[2]: The intra- and inter-laboratory **reproducibility** of the assay.

Repression (of genes): The interaction of specific molecules or molecular complexes with specific genes to prevent their expression (transcription of mRNA).

Scintillation counting: The measurement of radioactivity using a scintillation counter.

Screen/Screening Test[2]: A relatively rapid, simple test conducted for the purposes of a general classification of substances according to general categories of hazard. The results of a screen are generally used for preliminary decision-making and to set priorities for more definitive tests. A screening test may have a truncated response range (e.g., provides a qualitative response only).

Sensitivity[2]: The proportion of all positive substances that are correctly classified as positive in a test.

Solvent control: An untreated sample containing all components of a test system, including the solvent, that is processed with treated samples and other control samples to determine whether the solvent interacts with the test system.

Specificity[2]: The proportion of all negative substances that are correctly classified as negative in a test.

Stereospecific: Refers to the orientation of atoms within a molecule. The specific orientation of some atoms can affect the chemical reactivity of the molecule.

Steroidogenesis assay: Measurement of the ability of chemicals to inhibit steroid hormone biosynthesis in testicular tissue or cells *in vitro*.

Sulfhydryl: Chemical containing sulfur in the form of a -SH group.

Test battery: A series of tests, usually performed at the same time or in close sequence. Each test in the battery usually measures a different component of a multifactorial toxic effect, or a mechanistically-related effect.

Tier 1 assay for endocrine disruptors: An assay that is a component of the EDSP screening battery of tests.

Tier 1 battery for endocrine disruptors: Defined by the EDSP as a series of *in vitro* and *in vivo* tests to determine the ability of substances to interact with the endocrine system.

Tier 2 assay for endocrine disruptors: An assay that is a component of the EDSP testing battery.

Tier 2 battery for endocrine disruptors: Defined by the EDSP as a series of *in vivo* tests designed to confirm the endocrine disrupting ability of substances in laboratory animals and wildlife species.

Transcriptional activation: The initiation of mRNA synthesis in a gene in response to a specific chemical signal, such as an estrogen-estrogen receptor complex.

Transcriptional regulatory protein: A protein that binds to a specific DNA sequence resulting in a change in the regulation of mRNA synthesis.

Uterotrophic assay: Measures the ability of a substance to cause uterine enlargement in an immature or ovariectomized rat or mouse. A test method for potential estrogenicity and anti-estrogenicity.

Valid method[2]: A method determined to be acceptable for a specific use.

Validated method[2]: A method for which the reliability and relevance for a specific purpose has been established.

Validation[2]: The process by which the reliability and relevance of a procedure for a specific purpose are established.

Vector: A small segment of DNA (frequently a plasmid or viral DNA) that is used to carry a foreign gene or DNA sequence into a cell's nucleus.

Weight of evidence (process): The strengths and weaknesses of a collection of information are used as the basis for a conclusion that may not be evident from the individual data.

Xenobiotic: A substance that is not produced by the organism of interest.

Zinc finger motif: A configuration of a DNA-binding protein that resembles a finger and binds a zinc ion for its activity.

Appendix A
In Vitro ER Binding Assays

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Appendix A1

Assays Using Rat Uterine Cytosol

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Assays Using Rat Uterine Cytosol

Reference	Acton et al. (1983)	Allen et al. (1980)	Anstead et al. (1989)
Preparation of receptor			
Species/strain from which receptor obtained	Rats (otherwise unspecified)	Rats (otherwise unspecified)	Rats (otherwise unspecified)
Age of animals	Mature	n.p.	n.p.
Source of receptor	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol
When ovariectomized	n.p.	estradiol benzoate treated 3 x 0.16 µg	n.p.
Buffer for preparation of cytosol	n.p.	TED (10 mM Tris, 1.5 mM EDTA, 0.5 mM dithiothreitol)	n.p.
Dilution of tissue with buffer	n.p.	8 uteri in 4 mL TED buffer	n.p.
Protein concentration of cytosol	n.p.	n.p.	n.p.
Competitive binding assay			
Volume and concentration of radiolabelled 17β -estradiol	volume n.p.; 1 nM	50 µL; $7x10^{-9}$ mol/L	n.p.
Specific activity of radioligand	n.p.	58 Ci/mmol	n.p.
Solvent used to dissolve competing ligand	ethanol	TED buffer	n.p.
Concentration range of competing ligand	10^{-3} to 10 µg/mL	10^{-9} to $10^{-5.5}$ mol/L	10^{-3} to 10 µg/mL
Volume of ER prep used	n.p.	150 µL	n.p.
No. of replicates	n.p.	n.p.	n.p.
No. of times assay repeated	n.p.	n.p.	n.p.
Time of incubation	16 hours	30 mins	n.p.
Temperature of incubation	3ºC	30ºC	n.p.
Measure of nonspecific binding(y/n) and concentration	y, 10^{-3} to 10 µg/mL	n.p.	n.p.
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	Dextran-coated charcoal	200 µL dextran-coated charcoal (250mg% Norit A, 25mg% dextran in TED buffer)	Dextran-coated charcoal
Incubation time and temperature	n.p.	20 min. in ice cold water	n.p.
Centrifugation speed	n.p.	2000xg	n.p.
Centrifugation time and temperature	n.p.	4ºC for 5 min.	n.p.
Data calculations			
Program or method used for calculating data	n.p.	n.p.	n.p.
Data plotted as	n.p.	% specific ct./min. to controls vs. Conc. ligand in incubate (mol/L)	n.p.
Data format in paper (*e.g.,* IC_{50}, K_i)		graphical	n.p.
Calculation of RBA	IC_{50} E_2/IC_{50} ligand (estimated)	IC_{50} E_2/IC_{50} competitor x100	n.p.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Rat Uterine Cytosol

Reference	Ashby et al. (1999)	Blair et al. (2000)	Connor et al. (1997)
Preparation of receptor			
Species/strain from which receptor obtained	AP Rats	Sprague Dawley rats	Sprague Dawley rats
Age of animals	21-25 days old	245±18 days old	24 days
Source of receptor	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol
When ovariectomized	n.p.	10 days prior to sacrifice	n.p.
Buffer for preparation of cytosol	TEGM (10 mM Tris, 1.5 mM EDTA, $MgCl_2$ 3 mM, 10% glycerol, pH 7.6)	TEDG (10 mM Tris, 1.5 mM EDTA, 10 mM D, 10% glycerol, pH 7.4)	TESHMo (10 mM Tris-HCl, pH 7.4; 1.5 mM EDTA, 15 mM thioglycerol; 10 mM sodium molybdate)
Dilution of tissue with buffer	50 mg/mL buffer	100 mg/mL buffer	50 mg/mL buffer
Protein concentration of cytosol	n.p.	n.p.	n.p.
Competitive binding assay			
Volume and concentration of radiolabelled 17β -estradiol	volume n.p.; 5 nM - 500 µM	10 µL; 1 nM	volume n.p.; 10 nM
Specific activity of radioligand	n.p.	141 Ci/mmol	130 Ci/mmol
Solvent used to dissolve competing ligand	n.p.	100% ethanol	n.p.
Concentration range of competing ligand	5 nM - 500 µM	n.p.	1 mM - 0.1 µM
Volume of ER prep used	100 µL	50 µL	n.p.
No. of replicates	n.p.	2	3
No. of times assay repeated	n.p.	2	n.p.
Time of incubation	18 hours	20 hours	8 hours
Temperature of incubation	4ºC	4ºC	4ºC
Measure of nonspecific binding(y/n) and concentration	n.p.	y	n.p.
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	Hydroxyapatite in TEGM buffer	750 µl cold hydroxyapatite in 50mM Tris pH 7.4	0.1 volume Dextran-coated charcoal
Incubation time and temperature	n.p.	20 min. at 4ºC	n.p.
Centrifugation speed	n.p.	600xg	8000xg
Centrifugation time and temperature	n.p.	4ºC for 5 min.	10 min.; temp. n.p.
Data calculations			
Program or method used for calculating data	n.p.	n.p.	n.p.
Data plotted as	% Control vs. Concentration (M)	% [^{3}H]-E_2 bound vs. Competitor concentration (M)	n.p.
Data format in paper (e.g., IC_{50}, K_i)	IC_{50}	IC_{50}	IC_{50}
Calculation of RBA	n.p.	IC_{50} E_2/IC_{50} competitor x 100	IC_{50} E_2/IC_{50} competitor

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Rat Uterine Cytosol

Reference	Elsby et al. (2000)	Fang et al. (2001)	Gabbard and Segaloff (1983)
Preparation of receptor			
Species/strain from which receptor obtained	AP rats	Sprague Dawley rats	AXC rats
Age of animals	21-25 days old	245±18 days old	Mature
Source of receptor	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol
When ovariectomized	n.p.	10 days prior to sacrifice	5 days prior to sacrifice
Buffer for preparation of cytosol	TEGM (10 mM Tris, 1.5 mM EDTA, $MgCl_2$ 3 mM, 10% glycerol, pH 7.6)	TEDG (10 mMTris, 1.5 mM EDTA, 10 mM D, 10% glycerol, pH 7.4)	1.5 mM Tris, 1.0 mM EDTA, 20 mM sodium molybdate, pH 7.4
Dilution of tissue with buffer	50 mg/mL buffer	17 mg/mL	10 mg/mL buffer
Protein concentration of cytosol	n.p.	n.p.	6 mg/mL
Competitive binding assay			
Volume and concentration of radiolabelled 17β -estradiol	volume n.p.; 5 nM - 500 µM	10 µL; 1 nM	n.p.
Specific activity of radioligand	111 Ci/mmol	141 Ci/mmol	53 Ci/mmol
Solvent used to dissolve competing ligand	n.p.	100% ethanol	ethanol
Concentration range of competing ligand	5 nM - 500 µM	n.p.	1 nM to 1 µM
Volume of ER prep used	100 µL	50 µL	100 µL
No. of replicates	n.p.	2	3
No. of times assay repeated	n.p.	2	n.p.
Time of incubation	18 hours	20 hours	2 hours
Temperature of incubation	4°C	4°C	4°C
Measure of nonspecific binding(y/n) and concentration	n.p.	n.p.	n.p.
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	250 µL 60% Hydroxyapatite in TEGM buffer	750 µl cold hydroxyapatite in 50mM Tris pH 7.4	Dextran-coated charcoal (Norite-A + dextran + human gamma globulin)
Incubation time and temperature	n.p.	20 min. at 4°C	15 min. at 4°C
Centrifugation speed	1000xg	600xg	3500xg
Centrifugation time and temperature	10 min. at room temp.	4°C for 5 min.	10 min. at 0°C
Data calculations			
Program or method used for calculating data	n.p.	n.p.	n.p.
Data plotted as	% Control vs. Concentration (M)	n.p.	% Bound radioactivity vs. Log concentration
Data format in paper (*e.g.,* IC_{50}, K_i)	IC_{50}	RBA	relative displacing activity (RDA)
Calculation of RBA	IC_{50} E_2/IC_{50} competitor x 100	IC_{50} E_2/IC_{50} competitor x 100	IC_{50} E_2/IC_{50} competitor x 100

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Rat Uterine Cytosol

Reference	Jaimez et al. (2000)	Jordan et al. (1986)	Laws et al. (1996)
Preparation of receptor			
Species/strain from which receptor obtained	Rats (otherwise unspecified)	Sprague Dawley rats	Long Evans rats
Age of animals	Immature	18-21 days	Adult (60 days)
Source of receptor	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol
When ovariectomized	n.p.	n.p.	11 days prior to sacrifice
Buffer for preparation of cytosol	TEDM (20mM Tris-HCl, 1.5 mM EDTA, 0.25 mM dithiothreitol, 10 µg/mL leupeptine, 10% glycerol, pH 7.4)	TED (10 mMTris, 1.5 mM EDTA, 0.5 mM dithiothourseitol, pH 7.4)	TE-G30%-MTG (50 mM Tris, 0.9 mM EDTA, 30% (v/v) glycerol, 0.15% (v/v) monothioglycerol, pH 7.4)
Dilution of tissue with buffer	1:6 (w/v) tissue to buffer ratio	2 uteri/mL buffer	50 mg/mL buffer
Protein concentration of cytosol	n.p.	n.p.	n.p.
Competitive binding assay			
Volume and concentration of radiolabelled 17β -estradiol	volume n.p.; 1 nM	100 µL; $5x10^{-9}$ mol/L	volume n.p.; 1 nM
Specific activity of radioligand	n.p.	51 Ci/mmol	111Ci/nmol
Solvent used to dissolve competing ligand	n.p.	ethanol	20% glycerol/ethanol; TE-G30%-MTG buffer
Concentration range of competing ligand	n.p.	n.p.	0.0001-1000 µM
Volume of ER prep used	n.p.	200 µL	200 µL
No. of replicates	n.p.	n.p.	n.p.
No. of times assay repeated	n.p.	n.p.	n.p.
Time of incubation	18 hours	18 hours or 30 min.	30 min.
Temperature of incubation	4ºC	30ºC or 4ºC	30ºC
Measure of nonspecific binding(y/n) and concentration	n.p.	y	y
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	Dextran-coated charcoal (250 mg Norite-A + 25 mg Dextran T-70 in 100 mL TEDM buffer)	Dextran-coated charcoal (0.25% Norit A and 0.025% dextran in TED buffer)	250 µL 60% hydroxyapatite in TEG-MTG buffer
Incubation time and temperature	n.p.	20 min. at 4ºC	15 min. at room temp.
Centrifugation speed	800xg	2000xg	1000xg
Centrifugation time and temperature	15 min. at 4ºC	10 min. at 4ºC	10 min. at 4ºC
Data calculations			
Program or method used for calculating data	n.p.	n.p.	Graph Pad Prism
Data plotted as	n.p.	% specific [^{3}H]-E_2 binding in controls vs. log concentration (mol/L)	% Specific binding vs. Competitor (M)
Data format in paper (*e.g.,* IC_{50}, K_i)	RBA	IC_{50}	K_i calculated from EC_{50}
Calculation of RBA	n.p.	IC_{50} E_2/IC_{50} competitor x 100	n.p.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Rat Uterine Cytosol

Reference	Laws et al. (2000)	Leibl and Spona (1982)	Liu et al. (1994)
Preparation of receptor			
Species/strain from which receptor obtained	Long Evans rats	Sprague Dawley rats	Sprague Dawley rats
Age of animals	Adult (60 days)	Adult (60 - 80 days)	30 days
Source of receptor	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol
When ovariectomized	11 days prior to sacrifice	10 days prior to sacrifice	n.p.
Buffer for preparation of cytosol	TE-G30%-MTG (50 mM Tris, 0.9 mM EDTA, 30% (v/v) glycerol, 0.15% (v/v) monothioglycerol, pH 7.4)	TMK buffer (10 mM Tris, 1.5 mM MgCl2, 10 mM KCl, pH 7.2)	TEGD (10 mM Tris, 1.5 mM EDTA, 1 mM dithiothourseitol, 10% (v/v) glycerol)
Dilution of tissue with buffer	50 mg/mL buffer	5 uteri/4 mL buffer	n.p.
Protein concentration of cytosol	n.p.	n.p.	n.p.
Competitive binding assay			
Volume and concentration of radiolabelled 17β *-estradiol*	volume n.p.; 1 nM	volume n.p.; 1 nM	volume n.p.; 1 nM
Specific activity of radioligand	111Ci/nmol	58 Ci/mmol	147 Ci/mmol
Solvent used to dissolve competing ligand	20% glycerol/ethanol; TE-G30%-MTG buffer	ethanol	n.p.
Concentration range of competing ligand	0.0001-1000 µM	1 pM -1 µM	1 nM - 10 µM
Volume of ER prep used	200 µL	n.p.	200 µg protein
No. of replicates	n.p.	2	n.p.
No. of times assay repeated	n.p.	n.p.	3
Time of incubation	30 min.	18 hours	2 hours
Temperature of incubation	30ºC	4ºC	22ºC
Measure of nonspecific binding(y/n) and concentration	y	n.p.	y
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	250 µL 60% hydroxyapatite in TEG-MTG buffer	0.25 mL dextran-coated charcoal (0.6% charcoal Norit A, 0.06% dextran T-60)	100 µL dextran-coated charcoal (5% charcoal and 0.5% dextran in TEGD buffer)
Incubation time and temperature	15 min. at room temp.	20 min., 4ºC	20 min., 4ºC
Centrifugation speed	1000xg	3000xg	1500xg
Centrifugation time and temperature	10 min. at 4ºC	10 min.; temp. n.p.	10 min. at 4ºC
Data calculations			
Program or method used for calculating data	Graph Pad Prism	n.p.	n.p.
Data plotted as	% Specific binding vs. Competitor (M)	% Bound vs. Concentration competitor	$[^3H]$-E_2 complex (%) vs. ligand (nM)
Data format in paper (*e.g.,* IC_{50}, K_i)	K_i calculated from EC_{50}	graphical	graphical (EC_{50} estimated)
Calculation of RBA	n.p.	n.p.	n.p.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Rat Uterine Cytosol

Reference	McBlain (1987)	Nelson et al. (1973)	Olea et al. (1996)
Preparation of receptor			
Species/strain from which receptor obtained	Sprague Dawley rats	Sprague Dawley rats	Rats (otherwise unspecified)
Age of animals	4-5 weeks	2-5 months	Immature
Source of receptor	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol
When ovariectomized	n.p.	n.p.	n.p.
Buffer for preparation of cytosol	(10 mMTris, 1.5 mM EDTA, 12 mM monothioglycerol, 10 mM sodium molybdate, 10% (v/v) glycerol, pH 7.4)	10 mM Tris-HCL+1.5 mM EDTA, pH 7.4	Phosphate buffer
Dilution of tissue with buffer	Uteri powdered under liquid N_2	Uteri from 3-4 mice in 10 mL	n.p.
Protein concentration of cytosol	n.p.	n.p.	2 mg/mL
Competitive binding assay			
Volume and concentration of radiolabelled 17β *-estradiol*	volume n.p.; 2 nM	volume n.p.; 2 nM	volume n.p.; 3 nM
Specific activity of radioligand	n.p.	48 Ci/mmole	103 Bq/mmol
Solvent used to dissolve competing ligand	ethanol (final conc. 1.5%)	absolute ethanol	ethanol
Concentration range of competing ligand	0.2 nM-20 µM	0.1 to 500 µM	0.1 nM to 100 µM
Volume of ER prep used	n.p.	400 µg protein	n.p.
No. of replicates	n.p.	n.p.	n.p.
No. of times assay repeated	n.p.	3	n.p.
Time of incubation	18 hours	1 hour	16 hours
Temperature of incubation	4°C	4°C	0-4°C
Measure of nonspecific binding(y/n) and concentration	y	n.p.	y
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	0.5 mL dextran-coated charcoal + 1 mg/mL BSA	0.5 mL activated charcoal and 0.5m% Dextran T40 in Tris/HCL	Dextran + charcoal
Incubation time and temperature	10 min.	15 min. at 4°C	n.p.
Centrifugation speed	12,800xg	2000xg for 5 min.	n.p.
Centrifugation time and temperature	5 min.; temp. n.p.	5 min.; temp. n.p.	n.p.
Data calculations			
Program or method used for calculating data	n.p.	n.p.	n.p.
Data plotted as	$[^3H]$-E_2 Bound (% of control) vs. Molar excess of competitor	% Inhibition of $[^3H]$-E_2 binding vs. Concentration (µM)	% Specific binding vs. Concentration (M)
Data format in paper (*e.g.,* IC_{50}, K_i)	IC_{25}, IC_{50}, K_i	graphical	graphical, RBA
Calculation of RBA	n.p.	n.p.	IC_{50} E_2/IC_{50} competitor x 100

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Rat Uterine Cytosol

Reference	Perez et al. (1998)	Qian and Abul-Hajj (1990)	Rijks et al. (1996)
Preparation of receptor			
Species/strain from which receptor obtained	Rats (otherwise unspecified)	Sprague Dawley rats	Sprague Dawley rats
Age of animals	Immature	Immature	Mature
Source of receptor	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol
When ovariectomized	n.p.	n.p.	n.p.
Buffer for preparation of cytosol	Phosphate buffer	n.p.	ER buffer (10 mMTris.HCl, 1.0 mM EDTA, 1 mM dithiothourseitol, 10 mM sodium molybdate, 0.25M sucrose, pH 7.4)
Dilution of tissue with buffer	n.p.	n.p.	200 mg/mL buffer
Protein concentration of cytosol	2 mg/mL	3.5 mg/mL	3-4 mg/mL
Competitive binding assay			
Volume and concentration of radiolabelled 17β -estradiol	volume n.p.; 3 nM	50 µl; concentration n.p.	50 µl; concentration n.p.
Specific activity of radioligand	103 Bq/mmol	91Ci/mmol	4.26 TBq/mmol
Solvent used to dissolve competing ligand	ethanol	n.p.	n.p.
Concentration range of competing ligand	10 pM to 100 µM	1 nM to 3 µM	10 pM to 2 µM
Volume of ER prep used	n.p.	150 µl	50 µL
No. of replicates	n.p.	2	n.p.
No. of times assay repeated	n.p.	n.p.	2
Time of incubation	16 hours	3 hours	18 hours
Temperature of incubation	4°C	4°C	4°C
Measure of nonspecific binding(y/n) and concentration	y	y	n.p.
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	Dextran + charcoal	Dextran-coated charcoal (10 mM Trizma base, 1.0 mM EDTA, 250 mM sucrose, 0.05% dextran, 0.5% charcoal)	n.p.
Incubation time and temperature	n.p.	15 min. 4°C	n.p.
Centrifugation speed	n.p.	2000xg	n.p.
Centrifugation time and temperature	n.p.	0°C for 5 min.	n.p.
Data calculations			
Program or method used for calculating data	n.p.	n.p.	LIGAND computer program
Data plotted as	% Specific binding vs. Concentration (M)	n.p.	% Bound vs. Concentration competitor (nM)
Data format in paper (*e.g.,* IC_{50}, K_i)	graphical, RBA	RBA	K_i
Calculation of RBA	IC_{50} E_2/IC_{50} competitor x 100	IC_{50} E_2/IC_{50} competitor x 100	K_i reference steroid/ K_i competitor x 100

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Rat Uterine Cytosol

Reference	Routledge et al. (1998)	Waller et al. (1996)	Zacharewski et al. (1998)
Preparation of receptor			
Species/strain from which receptor obtained	Rats (otherwise unspecified)	Long Evans rats	Sprague Dawley rats
Age of animals	8-10 weeks	Adult (60 days)	22 day old
Source of receptor	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol
When ovariectomized	n.p.	11 days prior to sacrifice	n.p.
Buffer for preparation of cytosol	TEGM (10 mMTris, 1.5 mM EDTA, 3 mM $MgCl_2$, 10% glycerol, pH 7.4)	TE-G30%-MTG (50 mM Tris, 0.9 mM EDTA, 30% (v/v) glycerol, 0.15% (v/v) monothioglycerol, pH 7.4)	TEGD buffer (10 mMTris base 1.5 mM EDTA, 1 mM dithiothourseitol, 10% glycerol, pH 7.6)
Dilution of tissue with buffer	50 mg/mL	50 mg/mL buffer	200 mg/mL buffer
Protein concentration of cytosol	n.p.	n.p.	2 mg/mL
Competitive binding assay			
Volume and concentration of radiolabelled 17β *-estradiol*	volume n.p.; 5 nM	volume n.p.; 1 nM	30 µL; 1 nM
Specific activity of radioligand	110 Ci/mmol	111Ci/nmol	84 Ci/mmol
Solvent used to dissolve competing ligand	n.p.	20% glycerol/ethanol; TE-G30%-MTG buffer	dimethyl sulfoxide
Concentration range of competing ligand	0.5 nM -500 µM	0.0001-1000 µM	1-1000 µM
Volume of ER prep used	100 µL	200 µL	240 µL
No. of replicates	2	n.p.	2
No. of times assay repeated	n.p.	n.p.	3
Time of incubation	18 hours	30 min.	30 min.
Temperature of incubation	4ºC	30ºC	30ºC
Measure of nonspecific binding(y/n) and concentration	n.p.	y	y, 30 µL
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	250 µL 60% hydroxyapatite	250 µL 60% hydroxyapatite in TEG-MTG buffer	125 µL 60% hydroxyapatite in TEGD buffer
Incubation time and temperature	n.p.	15 min. at room temp.	n.p.
Centrifugation speed	1,000xg	1000xg	n.p.
Centrifugation time and temperature	10 min.	10 min. at room temp.	n.p.
Data calculations			
Program or method used for calculating data	Ligand Competition Analysis Software (Lundon Software, Chagrin Falls, OH)	one side competitive binding curves (Graph Pad Prism)	n.p.
Data plotted as	% Control vs. Molarity	% Specific binding vs. Competitor (M)	$[^3H]$-E_2 Bound vs. Log concentration of unlabeled competitor (M)
Data format in paper (*e.g.,* IC_{50}, K_i)	graphical	K_i calculated from EC_{50}	IC_{50}
Calculation of RBA	n.p.	n.p.	n.p.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Appendix A2

Assays Using Mouse Uterine Cytosol

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Assays Using Mouse Uterine Cytosol

Reference	Chae et al. (1991)	Connor et al. (1997)	Fielden et al. (1997)	Korach et al. (1978)
Preparation of receptor				
Species or cell line from which receptor obtained	CD-1 (ICR) BR mice	B6C3F1 mice	CD-1 mice	CD-1 mice
Age of animals/cells	8-10 weeks	24 days	Shortly after weaning	26 days
Source of receptor	Uterus	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol	Cytosol
When ovariectomized	n.p.	n.p.	n.p.	n.p.
Buffer for preparation of cytosol	TEGM buffer (10 nM Tris, 1.5 mM EDTA, 10% glycerol, 3mM MgCl2, pH 7.6)	Ice cold TESHMo buffer (10 mM Tris-Cl, pH 7.4, 1.5 mM EDTA, 15 mM thioglycerol, 10mM sodium molybdate)	Ice cold TEGD buffer (10 mM tris base, 1.5 mM EDTA, 10% glycerol, 1 mM dithiothreitol, pH 7.6)	Ice cold Tris/EDTA/ glycerol
Dilution of tissue with buffer	50 mg tissue/mL buffer	50 mg tissue/mL buffer	50 mg tissue/ mL buffer	n.p.
Protein concentration of cytosol	n.p.	n.p.	2.0 mg/mL	2 mg/mL
Ammonium sulfate fractionation	n.a.	n.a.	n.a.	n.a.
Competitive binding assay				
Radioligand used	^{3}H-17β-estradiol	^{3}H-17β-estradiol	^{3}H-17β-estradiol	^{3}H-17β-estradiol
Volume, concentration of radioligand	vol n.p.; final conc 5 nM	vol n.p.; final conc 10 nM	30 µL; final conc 1 nM	vol n.p.; final conc 0.1 nM
Specific activity of radioligand	n.p.	130 Ci/mmol	130 Ci/mmol	110 Ci/mmol
Solvent used to dissolve competing ligand	n.p.	n.p.	dimethyl sulfoxide	n.p.
Concentration range of competing ligand	0.5 nM - 5 µM	n.p.	1 nM - 1000 µM	n.p.
Volume of ER prep used	100 µL	n.p.	240 µL	100 µL
No. of replicates	2	1	2	n.p.
No. of times assay repeated	3	3	n.p.	n.p.
Incubation time and temperature	18 hours; 4º C	8 hours; 4º C	30º C for 30 min; then cooled to 4º C	18 hours; 0-4º C
Measured nonspecific binding (y/n)	y	n.p.	y	n.p.
Separation of ligand				
Volume and type of slurry (hydroxyapatite, charcoal, protamine sulfate)	250 µl hydroxyapatite in TEGM	0.1 vol dextran-coated charcoal	125 µl 60% (v/v) hydroxylapatite suspension in TEGD buffer	Protamine sulfate
Incubation time and temperature	n.p.	On ice	n.p.	10 min/4ºC
Centrifugation speed	1,000 g	8,000 g	n.p.	2,000 g
Centrifugation time and temperature	10 min; temp n.p.	10 min; temp n.p.	n.p.	10 min/4ºC
Data calculations				
Program or method used for calculating data	Ligand Competition Analysis Software by EMF	n.p.		n.p.
Data plotted as	% receptor bound vs. molar excess competitor	No plot	Percent specific binding of [^{3}H]E_2 vs. log concentration of competitor (M)	n.p.
Data format in paper (e.g., IC_{50}, K_i)	C_{50} and RBA	IC_{50}	IC_{50}	RBA
Calculation of RBA	n.p.	IC_{50} E_2/IC_{50} competitor x100	n.p.	IC_{50} E_2/IC_{50} competitor x100

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Mouse Uterine Cytosol

Reference	Korach et al. (1979)	Korach et al. (1985)	Korach et al. (1988)	Korach et al. (1989)
Preparation of receptor				
Species or cell line from which receptor obtained	CD-1 mice	CD-1 (ICR) BR mice	CD-1 mice	CD-1 (ICR) BR mice
Age of animals/cells	On or before 24 days	n.p.	On or before 24 days	n.p.
Source of receptor	Uterus	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol	Cytosol
When ovariectomized	5 days prior to sacrifice	7 days prior to sacrifice	5 days prior to sacrifice	7 days prior to sacrifice
Buffer for preparation of cytosol	Ice cold TEG buffer (10 mM Tris, 1.5 mM disodium EDTA, 10% glycerol, pH 8.0)	Ice cold TEGM buffer (pH 8.0, 4ºC, 10 mM Tris, 1.5 mM disodium EDTA, 10% glycerol, 3mM MgCl2)	TEG buffer (10 mM Tris, 1.5 mM EDTA, 10% glycerol, pH 7.6)	Ice cold TEGM buffer (pH 8.0, 4ºC, 10 mM Tris, 1.5 mM disodium EDTA, 10% glycerol, 3mM MgCl2)
Dilution of tissue with buffer	n.p.	n.p.	75 mg wet weight/mL buffer	50 mg tissue/mL buffer
Protein concentration of cytosol	1 mg/mL approximate	1 mg/mL approximate	n.p.	1.7 mg/mL
Ammonium sulfate fractionation	n.a.	n.a.	Cytosolic ER was enriched by a 0-40% ammonium sulfate fractionation	n.a.
Competitive binding assay				
Radioligand used	^{3}H-17β-estradiol	^{3}H-17β-estradiol	^{3}H-17β-estradiol	^{3}H-17β-estradiol
Volume, concentration of radioligand	vol n.p.; final conc 5 nM	vol n.p.; final conc 5 nM	n.p.	vol n.p.; final conc 5 nM
Specific activity of radioligand	110 Ci/mmol	98 Ci/mmol	n.p.	98 Ci/mmol
Solvent used to dissolve competing ligand	n.p.	n.p.	n.p.	n.p.
Concentration range of competing ligand	1 nM - 2.5 µM	1 nM - 2.5 µM	n.p.	0.5 - 500 mM
Volume of ER prep used	100 µL	100 µL	n.p.	200 µL
No. of replicates	3	n.p.	n.p.	
No. of times assay repeated	5 or more	n.p.	n.p.	4
Incubation time and temperature	18 hours; 4º C	18 hours; 4º C	n.p.	18 hours; 4º C
Measured nonspecific binding (y/n)	n.p.	n.p.	y	n.p.
Separation of ligand				
Volume and type of slurry (hydroxyapatite, charcoal, protamine sulfate)	Protamine sulfate	Protamine sulfate	Hydroxylapatite adsorption	Hydroxylapatite adsorption
Incubation time and temperature	10 min/4ºC	10 min/4ºC	10 min/4ºC	n.p.
Centrifugation speed	2,000 g	2,000 g	2,000 g	n.p.
Centrifugation time and temperature	10 min/4ºC	10 min/4ºC	10 min/4ºC	n.p.
Data calculations				
Program or method used for calculating data	n.p.	n.p.	n.p.	n.p.
Data plotted as	Semi-log plot of % Receptor bound vs. log molar excess of unlabeled competitor	Scatchard plot (bound/unbound vs. bound)	Semi-log plot of % Receptor bound vs. log molar excess of unlabeled competitor	% receptor bound vs. molar excess competitor
Data format in paper (*e.g.,* IC_{50}, K_i)	IC_{50}	C_{50} (molar equivalents of unlabeled competitor required to occupy 50% of the receptor binding sites)	C_{50}	C_{50}
Calculation of RBA	n.p.	n.p.	n.p.	n.p.

Abbreviations: n.p. = not provided;
n.a. = not applicable; RBA = relative binding affinity

Assays Using Mouse Uterine Cytosol

Reference	Korach (1979)	Matthews et al. (2001)	Ramamoorthy et al. (1997a)	Ramamoorthy et al. (1997b)
Preparation of receptor				
Species or cell line from which receptor obtained	CD-1 mice	CD-1 mice	B6C3F1 mice	B6C3F1 mice
Age of animals/cells	24 days	21 days	24 days	24 days
Source of receptor	Uterus	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol	Cytosol
When ovariectomized	5 days prior to sacrifice	n.p.	n.p.	n.p.
Buffer for preparation of cytosol	Ice cold TE buffer(0.01 M Tris, .0015 M disodium EDTA, pH 8 or 7.4)	TEGD (10 nM Tris base, 1.5 mM EDTA, 10% glycerol, 1.0 mM DTT, pH 7.6)	Ice cold TESHMo buffer (10 mM Tris-Cl, pH 7.4, 1.5 mM EDTA, 15 mM thio-glycerol, 10mM sodium molybdate)	Ice cold TESHMo buffer (10 mM Tris-Cl, pH 7.4, 1.5 mM EDTA, 15 mM thio-glycerol, 10mM sodium molybdate)
Dilution of tissue with buffer	n.p.	50 mg tissue/ mL buffer	50 mg tissue/mL buffer	50 mg tissue/mL buffer
Protein concentration of cytosol	1 mg/mL approximate	2.0 mg/mL	n.p.	n.p.
Ammonium sulfate fractionation	n.a.	n.a.	n.a.	n.a.
Competitive binding assay				
Radioligand used	^{3}H-17β-estradiol	^{3}H-17β-estradiol	^{3}H-17β-estradiol	^{3}H-17β-estradiol
Volume, concentration of radioligand	vol n.p.; final conc 10 nM	5 µL; final conc 2.5 nM	n.p.	vol n.p.; final conc 10 nM
Specific activity of radioligand	110 Ci/mmol	n.p.	130 Ci/mmol	n.p.
Solvent used to dissolve competing ligand	n.p.	dimethyl sulfoxide	n.p.	n.p.
Concentration range of competing ligand	0.1 - 1000-fold molar excess	n.p.	10 nM - 10 µM	n.p.
Volume of ER prep used	100 µL	240 µL	n.p.	n.p.
No. of replicates	n.p.	4	n.p.	n.p.
No. of times assay repeated	n.p.	n.p.	n.p.	n.p.
Incubation time and temperature	18 hours; 4º C	2 hours; 30º C	8 hours; 4º C	16-18 hours; 4º C
Measured nonspecific binding (y/n)	n.p.	n.p.	n.p.	n.p.
Separation of ligand				
Volume and type of slurry (hydroxyapatite, charcoal, protamine sulfate)	Protamine sulfate	96-well filter plate and vacuum pump harvester	0.1 vol DCC suspension (0.5% dextran: 5% charcoal, wt/vol in TESHMo)	0.1 vol DCC suspension (0.5% dextran: 5% charcoal, wt/vol in TESHMo)
Incubation time and temperature	10 min/4ºC	Samples dried under suction for 30 sec; Filter plates sealed. Scintillation cocktail added to each well.	10 min; temp n.p.	10 min; temp n.p.
Centrifugation speed	n.p.	n.a.	5,000 g	5,000 g
Centrifugation time and temperature	10 min/4ºC	n.a.	10 min; temp n.p.	10 min; temp n.p.
Data calculations				
Program or method used for calculating data	n.p.	GraphPad Prism 3.0 software	n.p.	n.p.
Data plotted as	Scatchard plot	Percent specific binding of [^{3}H]E_2 vs. log competitor concentration	%[^{3}H]E_2 bound vs. log [M]	DPM vs. log [M]
Data format in paper (*e.g.,* IC_{50}, K_i)	C_{50} (molar excess of unlabeled competitor which inhibits 50% specific receptor binding)	IC_{50}	n.p.	IC_{50}
Calculation of RBA	n.p.	n.p.	n.p.	n.p.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Mouse Uterine Cytosol

Reference	Shelby et al. (1996)	Waller et al. (1996)
Preparation of receptor		
Species or cell line from which receptor obtained	CD-1 BR mice	CD-1 mice
Age of animals/cells	Either 8-10 weeks or 12-14 weeks	On or before 24 days
Source of receptor	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol
When ovariectomized	2 weeks prior to sacrifice	5 days prior to sacrifice
Buffer for preparation of cytosol	TEGM buffer (10 nM Tris, 1.5 mM EDTA, 10% glycerol, 3mM MgCl2, pH 7.6)	TEG buffer (10 mM Tris, 1.5 mM EDTA, 10% glycerol, pH 7.6)
Dilution of tissue with buffer	50 mg tissue/mL buffer	75 mg wet weight/mL buffer
Protein concentration of cytosol	n.p.	n.p.
Ammonium sulfate fractionation	n.a.	Cytosolic ER was enriched by a 0-40% ammonium sulfate fractionation for some binding experiments
Competitive binding assay		
Radioligand used	^{3}H-17β-estradiol	^{3}H-17β-estradiol
Volume, concentration of radioligand	final conc 5 nM	final conc 5 nM
Specific activity of radioligand	n.p.	n.p.
Solvent used to dissolve competing ligand	n.p.	n.p.
Concentration range of competing ligand	0.5 nM - 5 µM	2.5 nM - 25 µM
Volume of ER prep used	100 µL	100 µL
No. of replicates	2	n.p.
No. of times assay repeated	2	n.p.
Incubation time and temperature	18 hours; 4º C	18 hours; 4º C
Measured nonspecific binding (y/n)	n.p.	n.p.
Separation of ligand		
Volume and type of slurry (hydroxyapatite, charcoal, protamine sulfate)	250 µL 60% hydroxyapatite in TEGM buffer (10 nM Tris, 1.5 mM EDTA, 10% glycerol, 3mM MgCl2, pH 7.6)	Hydroxylapatite adsorption
Incubation time and temperature	n.p.	10 min/4ºC
Centrifugation speed	1,000 g	2,000 g
Centrifugation time and temperature	10 min; temp n.p.	10 min/4ºC
Data calculations		
Program or method used for calculating data	n.p.	n.p.
Data plotted as	Semi-log plot of % Receptor bound vs. Log competitor concentration (M)	n.p.
Data format in paper (*e.g.,* IC_{50}, K_i)	n.p.	n.p.
Calculation of RBA	n.p.	n.p.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Appendix A3

Assays Using Human ERα and ERβ

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Assays Using Human ERα and ERβ

Reference	Arcaro et al. (1999)	Arcaro et al. (2000)	Fertuck et al. (2001)
Preparation of receptor			
Species and subtype of receptor	human ER alpha and human ER beta	human ER alpha	human ER beta
Source of receptor	n.p.	n.p.	PanVera
Whole, truncated, recombinant, or chimeric	recombinant	recombinant	recombinant
Buffer for isolation of receptor	n.a.	n.a.	TEDG (10 mM Tris, 1.5 mM EDTA, 1 mM DDT, 10% glycerol containing 1 mg/mL BSA pH 7.6)
Protein concentration	1.2 nM	1.2 nM	n.p.
Competitive binding assay			
Radioligand used	^{3}H-17β-estradiol	^{3}H-17β-estradiol	^{3}H-17β-estradiol
Concentration of radioligand	2.5 nM	2.5 nM	10 pM - 1 μM
Solvent used to dissolve ligand	n.p.	n.p.	DMSO
Concentration range of competing ligand	5 nM - 100 μM	0.1 μM - 10 μM	60 nM - 20μM
Number of replicates	3	3	4
Number of times assay repeated	3	2	3
Time of incubation	4 hours	4 hours	24 hours
Temperature of incubation	room temperature	room temperature	4°C
Nonspecific binding measured (y/n)	y	y	n.p.
Separation of ligand			
Type of slurry	hydroxyapatite	hydroxyapatite	n.p.
Incubation time and temperature	15 min; n.p.	15 min; n.p.	n.p.
Centrifugation time and temperature	20 min; n.p.	20 min; n.p.	n.p.
Data calculations			
Program or method used for calculating data	SigmaPlot	SigmaPlot	Nonlinear regression using Graphpad Prism 3.0
Data plotted as	% ^{3}H-E_2 bound vs. log M of ligand	% ^{3}H-E_2 bound vs. log M of ligand	Specific binding vs. log competitor conc.
Data format in paper (*e.g.,* IC_{50}, K_i)	IC_{50}	IC_{50}	IC_{50}
Calculation of RBA	n.p.	n.p.	IC_{50} E_2/IC_{50} competitor

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Human ERα and ERβ

Reference	Gaido et al. (1999)	Klotz et al. (1996)	Kraichely et al. (2000)
Preparation of receptor			
Species and subtype of receptor	human ER alpha and human ER beta	human ER alpha	human ER alpha and human ER beta
Source of receptor	PanVera	Produced in Sf9 insect cells using a baculovirus expression system	PanVera
Whole, truncated, recombinant, or chimeric	recombinant	recombinant	recombinant
Buffer for isolation of receptor	n.a.	n.p.	n.a.
Protein concentration	8 pmol/mL (alpha) 11 pmol/mL (beta)	0.4 nM	1.5 nM
Competitive binding assay			
Radioligand used	^{3}H-17β-estradiol	^{3}H-17β-estradiol	^{3}H-17β-estradiol
Concentration of radioligand	5 nM	2.5 nM	10 nM
Solvent used to dissolve ligand	n.p.	dimethyl sulfoxide or ethanol	n.p.
Concentration range of competing ligand	0.1 nM - 10 µM	10 nM - 100 µM	n.p.
Number of replicates	3	3	n.p.
Number of times assay repeated	3	2	n.p.
Time of incubation	overnight	1 hour	18 hours
Temperature of incubation	4ºC	25ºC	0ºC
Nonspecific binding measured (y/n)	n.p.	y	n.p.
Separation of ligand			
Type of slurry	hydroxyapatite	5% activated charcoal/0.5% dextran	hydroxyapatite
Incubation time and temperature	30 min; 4ºC	10 min; 4ºC	15 min; 0ºC
Centrifugation time and temperature	10 min; n.p.	3 min; n.p.	Washed 3X with 1 mL of 0.05 M Tris, pH 7.3 buffer
Data calculations			
Program or method used for calculating data	GraphPad Prism software	n.p.	n.p.
Data plotted as	% Binding vs. log dose (M)	% ^{3}H-E_2 bound vs. [ligand] in nM	no plot of data reported
Data format in paper (*e.g.,* IC_{50}, K_i)	RBA	IC_{50}	RBA
Calculation of RBA	IC_{50} E_2/IC_{50} competitor	n.p.	n.p.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Human ERα and ERβ

Reference	Meyers et al. (1999)	Sun et al. (1999)	Sun et al. (1999)
Preparation of receptor			
Species and subtype of receptor	human ER alpha and human ER beta	human ER alpha ligand binding domain	human ER beta ligand binding domain
Source of receptor	PanVera	expressed in E coli using pET15b vector	expressed in E coli using pET15b vector
Whole, truncated, recombinant, or chimeric	recombinant	truncated (amino acids 304 - 554)	truncated (amino acids 256 - 505)
Buffer for isolation of receptor	n.a.	50 mM Tris buffer, pH 7.5, 10% glycerol, 0.1 mM butylated hydroxyanisole, 10 mM mercaptoethanol	50 mM Tris buffer, pH 7.5, 10% glycerol, 0.1 mM butylated hydroxyanisole, 10 mM mercaptoethanol
Protein concentration	1.5 nM	n.p.	n.p.
Competitive binding assay			
Radioligand used	^{3}H-17β-estradiol	^{3}H-17β-estradiol	^{3}H-17β-estradiol
Concentration of radioligand	10 nM	10 nM	10 nM
Solvent used to dissolve ligand	n.p.	n.p.	n.p.
Concentration range of competing ligand	n.p.	n.p.	n.p.
Number of replicates	2	n.p.	n.p.
Number of times assay repeated	n.p.	n.p.	n.p.
Time of incubation	18 - 24 hours	18 hours	18 hours
Temperature of incubation	0ºC	0ºC	0ºC
Nonspecific binding measured (y/n)	n.p.	n.p.	n.p.
Separation of ligand			
Type of slurry	hydroxyapatite	hydroxylapatite	hydroxylapatite
Incubation time and temperature	15 min; 0ºC	15 min; 0ºC	15 min; 0ºC
Centrifugation time and temperature	Washed 3X with 1 mL of 0.05 M Tris, pH 7.3 buffer	Washed 3X with 1 mL of 0.05 M Tris, pH 7.3 buffer	Washed 3X with 1 mL of 0.05 M Tris, pH 7.3 buffer
Data calculations			
Program or method used for calculating data	n.p.	Ki calculated using Cheng-Prusoff equation	Ki calculated using Cheng-Prusoff equation
Data plotted as	no plot of data reported	no plot of data reported	no plot of data reported
Data format in paper (*e.g.,* IC_{50}, K_i)	RBA	IC_{50} (not reported), Ki, and RBA	IC_{50} (not reported), Ki, and RBA
Calculation of RBA	n.p.	IC_{50} E_2/IC_{50} competitor x100	IC_{50} E_2/IC_{50} competitor x100

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Human ERα and ERβ

Reference	Vakharia and Gierthy (1999)	Vakharia and Gierthy (2000)
Preparation of receptor		
Species and subtype of receptor	human ER alpha	human ER alpha
Source of receptor	PanVera	PanVera
Whole, truncated, recombinant, or chimeric	recombinant	recombinant
Buffer for isolation of receptor	n.a.	n.a.
Protein concentration	1.2 nM	1.25 nM
Competitive binding assay		
Radioligand used	^{3}H-17β-estradiol	^{3}H-17β-estradiol
Concentration of radioligand	2.5 nM	2.5 nM
Solvent used to dissolve ligand	dimethyl sulfoxide	dimethyl sulfoxide
Concentration range of competing ligand	10 nM -1000 µM	50 nM - 50 µM
Number of replicates	3	3
Number of times assay repeated	n.p.	n.p.
Time of incubation	4 hours	4 hours
Temperature of incubation	room temperature	room temperature
Nonspecific binding measured (y/n)	y	y
Separation of ligand		
Type of slurry	hydroxyapatite	hydroxyapatite
Incubation time and temperature	n.p.	n.p.
Centrifugation time and temperature	10 min; n.p.	10 min; n.p.
Data calculations		
Program or method used for calculating data	Sigmaplot software	Sigmaplot software
Data plotted as	% ^{3}H-E_2 bound vs. [ligand] in nM	% ^{3}H-E_2 bound vs. [ligand] in nM
Data format in paper (*e.g.,* IC_{50}, K_i)	IC_{50}	IC_{50}
Calculation of RBA	n.a.	n.a.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Appendix A4

Assays Using Recombinant ERα and ERβ

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Assays Using Recombinant ERα and ERβ

Reference	Kuiper et al. (1997)	Kuiper et al. (1997)	Kuiper et al. (1998) [method a]
Preparation of receptor			
Species and subtype of receptor	rat ER beta	human ER alpha	human ER beta
Whole, truncated, recombinant, or chimeric	whole recombinant	whole recombinant	whole recombinant
Method of protein synthesis	*in vitro* using TnT-coupled reticulocyte lysate system	*in vitro* using TnT-coupled reticulocyte lysate system	Sf9 cells were infected with amplified baculovirus; infected cells were harvested after 48 h, and a nuclear fraction containing ER beta was obtained
RNA polymerase	T7-RNA polymerase	T7-RNA polymerase	n.a.
Reaction time or cell growth time	90 min reaction time	90 min reaction time	48 hours cell growth time
Reaction temperature	30ºC	30ºC	n.a.
Buffer for dilution of translation mixture or nuclear extract	20 mM HEPES, pH 7.9; 150 mM NaCl, 10% w/v glycerol, 1 mM EDTA, 6 mM Na_2MoO_4	20 mM HEPES, pH 7.9; 150 mM NaCl, 10% w/v glycerol, 1 mM EDTA, 6 mM Na_2MoO_4	17 mM K_2HPO_4, 3 mM KH_2PO_4, 40 mM KCl, 6 mM monothioglycerol, pH=7.6
Protein concentration	10 - 15 pM	10 - 15 pM	800 pM
Competitive binding assay			
Radioligand used	16α-[^{125}I]-estradiol	16α-[^{125}I]-estradiol	^{3}H-17β-estradiol
Concentration of radioligand	125 - 150 pM	125 - 150 pM	3 nM
Solvent used to dissolve ligand	dimethyl sulfoxide	dimethyl sulfoxide	dimethyl sulfoxide
Concentration range of competing ligand	0.001 - 100 µM	0.001 - 100 µM	n.p.
Volume of translation mixture or nuclear extract	2 µL	0.25 µL	200 µL nuclear extract per Scintistrip well
Time to allow adhesion of ER to Scintistrip wells	n.a.	n.a.	18 hours then washed 2X with buffer
Temperature to allow adhesion	n.a.	n.a.	ambient temperature
Number of replicates	2	2	n.p.
Number of times assay repeated	n.p.	n.p.	n.p.
Time of incubation	16 hours	16 hours	18 hours
Temperature of incubation	4ºC	4ºC	ambient temperature
Nonspecific binding measured (y/n)	y	y	n.p.
Separation of ligand			
Type of column	Gel filtration over Sephadex G-25 column	Gel filtration over Sephadex G-25 column	Solid-phase ligand binding using Scintistrip wells
Data calculations			
Program or method used for calculating data	Nonlinear 4-parameter logistic model to estimate IC_{50} and Cheng-Prusoff equation to calculate Ki	Nonlinear 4-parameter logistic model to estimate IC_{50} and Cheng-Prusoff equation to calculate Ki	Nonlinear 4-parameter logistic model to estimate IC_{50}
Data plotted as	% [^{125}I]-E_2 bound vs. log M of compound	% [^{125}I]-E_2 bound vs. log M of compound	no plot of data reported
Data format in paper (*e.g.,* IC_{50}, K_i)	IC_{50} (not reported), Ki and RBA	IC_{50} (not reported), Ki and RBA	IC_{50} (not reported) and RBA
Calculation of RBA	IC_{50} E_2/IC_{50} competitor x 100	IC_{50} E_2/IC_{50} competitor x 100	IC_{50} E_2/IC_{50} competitor x 100

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Recombinant ERα and ERβ

Reference	Kuiper et al. (1998) [method a]	Kuiper et al. (1998) [method b]
Preparation of receptor		
Species and subtype of receptor	human ER alpha	human ER beta
Whole, truncated, recombinant, or chimeric	whole recombinant	whole recombinant
Method of protein synthesis	Sf9 cells were infected with amplified baculovirus; infected cells were harvested after 48 h, and a nuclear fraction containing ER beta was obtained	Sf9 cells were infected with amplified baculovirus; infected cells were harvested after 48 h, and a nuclear fraction containing ER beta was obtained
RNA polymerase	n.a.	n.a.
Reaction time or cell growth time	48 hours cell growth time	48 hours cell growth time
Reaction temperature	n.a.	n.a.
Buffer for dilution of translation mixture or nuclear extract	17 mM K_2HPO_4, 3 mM KH_2PO_4, 40 mM KCl, 6 mM monothioglycerol, pH=7.6	20 mM HEPES, pH 7.5; 150 mM KCl, 1 mM EDTA, 6mM monothioglycerol, 8.7% (v/v) glycerol
Protein concentration	400 pM	0.3 - 0.4 nM
Competitive binding assay		
Radioligand used	^{3}H-17β-estradiol	^{3}H-17β-estradiol
Concentration of radioligand	3 nM	3 nM
Solvent used to dissolve ligand	dimethyl sulfoxide	dimethyl sulfoxide
Concentration range of competing ligand	n.p.	n.p.
Volume of translation mixture or nuclear extract	200 µL nuclear extract per Scintistrip well	n.p.
Time to allow adhesion of ER to Scintistrip wells	18 hours then washed 2X with buffer	n.a.
Temperature to allow adhesion	ambient temperature	n.a.
Number of replicates	n.p.	n.p.
Number of times assay repeated	n.p.	n.p.
Time of incubation	18 hours	18 - 20 hours
Temperature of incubation	ambient temperature	6°C
Nonspecific binding measured (y/n)	n.p.	n.p.
Separation of ligand		
Type of column	Solid-phase ligand binding using Scintistrip wells	Gel filtration over Sephadex G-25 column
Data calculations		
Program or method used for calculating data	Nonlinear 4-parameter logistic model to estimate IC_{50}	Nonlinear 4-parameter logistic model to estimate IC_{50}
Data plotted as	no plot of data reported	dpm bound radioligand vs. log M of compound
Data format in paper (e.g., IC_{50}, K_i)	IC_{50} (not reported) and RBA	IC_{50} (not reported) and RBA
Calculation of RBA	IC_{50} E_2/IC_{50} competitor x 100	IC_{50} E_2/IC_{50} competitor x 100

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Recombinant ERα and ERβ

Reference	Kuiper et al. (1998) [method b]	Morito et al. (2001)
Preparation of receptor		
Species and subtype of receptor	human ER alpha	human ER alpha; human ER beta
Whole, truncated, recombinant, or chimeric	whole recombinant	whole recombinant
Method of protein synthesis	Sf9 cells were infected with amplified baculovirus; infected cells were harvested after 48 h, and a nuclear fraction containing ER beta was obtained	Sf9 cells were infected with amplified baculovirus; harvested after 72 h and a cytosolic fraction made by sonication and centrifugation of the homogenate containing the ER alpha or ER beta
RNA polymerase	n.a.	n.a.
Reaction time or cell growth time	48 hours cell growth time	72 hours growth of cells
Reaction temperature	n.a.	28°C
Buffer for dilution of translation mixture or nuclear extract	20 mM HEPES, pH 7.5; 150 mM KCl, 1 mM EDTA, 6mM monothioglycerol, 8.7% (v/v) glycerol	40 mM Tris-HCL, pH 7.4, 0.5mM EDTA, 0.2M KCL, 10% (v/v) glycerol,1mM dithiothreitol, 1mM PMSF
Protein concentration	0.3 - 0.4 nM	36 µg/mL
Competitive binding assay		
Radioligand used	^{3}H-17β-estradiol	^{3}H-17β-estradiol
Concentration of radioligand	3 nM	2.5 pmoles
Solvent used to dissolve ligand	dimethyl sulfoxide	n.p.
Concentration range of competing ligand	n.p.	n.p.
Volume of translation mixture or nuclear extract	n.p.	5 µL
Time to allow adhesion of ER to Scintistrip wells	n.a.	n.a.
Temperature to allow adhesion	n.a.	n.a.
Number of replicates	n.p.	n.p.
Number of times assay repeated	n.p.	n.p.
Time of incubation	18 - 20 hours	16 hours
Temperature of incubation	6°C	0°C
Nonspecific binding measured (y/n)	n.p.	n.p.
Separation of ligand		
Type of column	Gel filtration over Sephadex G-25 column	0.5% activated charcoal and 0.05% dextran
Data calculations		
Program or method used for calculating data	Nonlinear 4-parameter logistic model to estimate IC_{50}	n.p.
Data plotted as	dpm bound radioligand vs. log M of compound	%^{3}H E_2 bound vs. fold excess of estradiol
Data format in paper (*e.g.,* IC_{50}, K_i)	IC_{50} (not reported) and RBA	Calculated IC_{50} by knowing that 1 fold increase was 5nM
Calculation of RBA	IC_{50} E_2/IC_{50} competitor x 100	IC_{50} E_2/IC_{50} competitor x 100

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

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Appendix A5

Assays Using Fluorescence Polarization

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Assays Using Fluorescent Polarization

Reference	Bolger et al. (1998)	Hanioka et al. (1999)	Hashimoto et al. (2000)
Preparation of receptor			
Species and subtype of receptor	human ERα	human ERα	human ERα
Source of receptor	PanVera	n.p.	n.p.
Whole, truncated, recombinant, or chimeric	recombinant	recombinant	recombinant
Buffer for assay of receptor	40 mM Tris-HCL, pH 7.5, 50 mM KCL, 5% glycerol, 10% dimethylformamide, 0.02% Na azide, 50µg/mL bovine gamma globulin	40 mM Tris-HCL, pH 7.5, 50 mM KCL, 5% glycerol, 10% dimethylformamide, 0.02% Na azide, 50 µg/mL bovine gamma globulin	40 mM Tris-HCL, pH 7.5, 50 mM KCL, 5% glycerol, 10% dimethylformamide, 0.02% Na azide, 50 µg/mL bovine gamma globulin
Protein concentration	n.p.	n.p.	n.p.
Competitive binding assay			
Ligand used	ES2	ES2	ES2
Concentration of estrogen	1 nM	1 nM	1 nM
Fluorescent ligand	FES1 ERα 13 nM, ERβ 10 nM	FES1 ERα 13 nM, ERβ 10 nM	FES1 ERα 13 nM, ERβ 10 nM
Concentration of fluorescent ligand	2 nM	2 nM	2 nM
Solvent used to dissolve competing ligand	10 mM ethanol	10 mM ethanol	10 mM ethanol
Concentration range of competing ligand	200 µM	200 µM	200 µM
Number of replicates	3	3	3
Number of times assay repeated	n.p.	n.p.	n.p.
Time of incubation	60 min	60 min	60 min
Temperature of incubation	room temp	room temp	room temp
Data calculations			
Fluorescence anisotropy	490 nm excitation; 530 nm emission filter	360 nm excitation; 535 nm emission filter	360 nm excitation; 530 nm emission filter
Program or method used for calculating data	Anisotropy converted to fraction bound	Nonlinear least squares regression	Anisotropy converted to percent inhibition
Data plotted as	Ligand bound=fraction bound x ligand conc.	Millipolarization vs. conc. of chemicals	Percent inhibition vs. competitor conc.
Data format in paper (*e.g.,* IC_{50}, K_i)	K_d	IC_{50}	Percent inhibition
Calculation of RBA	Nonlinear least squares regression	Nonlinear least squares regression	n.a.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Fluorescent Polarization

Reference	Nikov et al. (2000)	Nikov et al. (2001)	Parker et al. (2000)
Preparation of receptor			
Species and subtype of receptor	human ERα and ERβ	human ERα and ERβ	human ERα and ERβ
Source of receptor	PanVera	PanVera	PanVera
Whole, truncated, recombinant, or chimeric	recombinant	recombinant	n.p.
Buffer for assay of receptor	100 mM $KaPO_4$ pH 7.5; 100 µg/ml bovine gamma globulin, 0.02% sodium azide	100 mM $KaPO_4$ pH 7.5; 100 µg/ml bovine gamma globulin, 0.02% sodium azide	100 mM $KaPO_4$ pH 7.5; 100 µg/ml bovine gamma globulin, 0.02% sodium azide
Protein concentration	n.p.	n.p.	n.p.
Competitive binding assay			
Ligand used	ES2	ES2	ES2
Concentration of estrogen	1 nM	1 nM	1 nM
Fluorescent ligand	FES1	FES1	FES1 ERα 13 nM, ERβ 10 nM
Concentration of fluorescent ligand	n.p.	n.p.	n.p.
Solvent used to dissolve competing ligand	8 mM ethanol	8 mM ethanol	n.p.
Concentration range of competing ligand	n.p.	n.p.	n.p.
Number of replicates	n.p.	n.p.	n.p.
Number of times assay repeated	n.p.	n.p.	n.p.
Time of incubation	60 min	60 min	2 hours
Temperature of incubation	room temp	room temp	room temp
Data calculations			
Fluorescence anisotropy	490 nm excitation; 530 nm emission filter	490 nm excitation; 530 nm emission filter	483 nm excitation; 536 nm emission filter
Program or method used for calculating data	Nonlinear least squares regression, Prism, Graphpad (San Diego, CA)	Nonlinear binding isotherm	Nonlinear least squares regression, Prism, Graphpad (San Diego, CA)
Data plotted as	Percent inhibition vs. competitor conc.	Percent inhibition vs. competitor conc.	Millipolarization vs. conc. of chemicals
Data format in paper (*e.g.,* IC_{50}, K_i)	IC_{50}	IC_{50}	IC_{50}
Calculation of RBA	IC_{50} E_2/IC_{50} ligand X100	IC_{50} E_2/IC_{50} ligand X100	IC_{50} E_2/IC_{50} ligand X100

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Fluorescent Polarization

Reference	Saito et al. (2000)
Preparation of receptor	
Species and subtype of receptor	human ERα
Source of receptor	PanVera
Whole, truncated, recombinant, or chimeric	recombinant
Buffer for assay of receptor	40 mM Tris-HCL, pH 7.5, 50 mM KCL, 5% glycerol, 10% dimethylformamide, 0.02% Na azide, 50 µg/mL bovine gamma globulin
Protein concentration	n.p.
Competitive binding assay	
Ligand used	ES2
Concentration of estrogen	1 nM
Fluorescent ligand	FES1 ERα 13 nM, ERβ 10 nM
Concentration of fluorescent ligand	2 nM
Solvent used to dissolve competing ligand	10 mM ethanol
Concentration range of competing ligand	10 nM -10 µM
Number of replicates	3
Number of times assay repeated	n.p.
Time of incubation	60 min
Temperature of incubation	room temp
Data calculations	
Fluorescence anisotropy	490 nm excitation; 530 nm emission filter
Program or method used for calculating data	n.p.
Data plotted as	Percent inhibition vs. competitor conc.
Data format in paper (*e.g.,* IC_{50}, K_i)	n.p.
Calculation of RBA	n.p.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

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Appendix A6

Assays Using GST-ERdef Constructs

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Assays Using GST-ERdef Constructs

Reference	Fertuck et al. (2001)	Matthews and Zacharewski (2001)	Matthews et al. (2000)
Preparation of receptor			
Species and subtype of receptor	GST-hERα	GST-hERαdef, -aERdef, -cERdef, -rtERdef	GST-hERαdef, -aERdef, -cERdef, -rtERdef
Whole, truncated, recombinant, or chimeric	Recombinant, truncated	Recombinant, truncated fusion protein	Recombinant, truncated fusion protein
cDNA contained in	pGEX-hERαdef	pGEX-ERdef	pGEX-ERdef
Buffer for dilution of receptor	TEDG (10 mM Tris, 1.5 mM EDTA, 1 mM DTT, 10% glycerol containing 1mg/mL BSA, pH 7.6)	TEDG (10 mM Tris, 1.5 mM EDTA, 1 mM DTT, 10% glycerol containing 1mg/mL BSA, pH 7.6)	TEDG (10 mM Tris, 1.5 mM EDTA, 1 mM DTT, 10% glycerol containing 1mg/mL BSA, pH 7.6)
Protein concentration	1 mg/mL	1 mg/mL	1 mg/mL
Competitive binding assay			
Radioligand used and volume	5 µL of ^{3}H-E_2	5 µL of ^{3}H-E_2	5 µL of ^{3}H-E_2
Concentration of radioligand	2.5 nM	0.1 - 3.5 nM	0.1 - 3.5 nM
Solvent used to dissolve ligand	DMSO	5 µL DMSO	5 µL DMSO
Concentration range of competing ligand	60 nM - 20 µM	1 nM - 10 µM	1 nM - 10 µM
Volume of receptor	240 µL	240 µL	240 µL
Number of replicates	4	4	4
Number of times assay repeated	3	n.p.	n.p.
Time of incubation	2 hours	2 hours	2 hours
Temperature of incubation	4ºC	4ºC	4ºC
Nonspecific binding measured (y/n)	n.p.	y, 400x excess E_2	y, 400x excess E_2
Separation of ligand			
Type of column	n.p.	96-well filter plate and harvester of bound radioligand	96-well filter plate and harvester of bound radioligand
Washing solution	n.p.	TEG buffer (10mMTris, pH 7.6 1.5mM EDTA, 1mM DDT, 10% glycerol containing 1mg/mL BSA)	TEG buffer (10mMTris, pH 7.6 1.5mM EDTA, 1mM DDT, 10% glycerol containing 1mg/mL BSA)
Data calculations			
Program or method used for calculating data	Nonlinear regression	Nonlinear regression using Graphpad Prism 3.0	Nonlinear regression using Graphpad Prism 3.0
Data plotted as	Specific binding vs. log competitor conc.	Percent specific binding vs. log competitor conc.	Percent specific binding vs. log competitor conc.
Data format in paper (*e.g.,* IC_{50}, K_i)	IC_{50}	IC_{50}	IC_{50}
Calculation of RBA	IC_{50} E_2/IC_{50} ligand	IC_{50} E_2/IC_{50} ligand	IC_{50} E_2/IC_{50} ligand

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

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Appendix A7

Assays Using MCF-7 Cells and Cytosol

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Assays Using MCF-7 Cells and Cytosol

Reference	Arcaro et al. (1999)	Brooks et al. (1987)	Brooks et al. (1987)
Preparation of receptor			
Species or cell line	MCF-7 cells	MCF-7 cells	MCF-7 cells
Whole cells/cell homogenate	whole cells	homogenate	whole cells
Serum source	5% calf serum	10% calf serum	n.p.
Serum stripping method	n.p.	n.p.	n.p.
Residual E_2 in serum	n.p.	n.p.	n.p.
No. of treated cells	n.p.	n.p.	n.p.
Buffer for preparation of cell homogenate or cytosol	n.p.	Tris-EDTA + reducing agent, pH 7.4	n.p.
Protein concentration of cytosol	n.p.	n.p.	n.p.
Competitive binding assay			
Volume and concentration of ^{3}H-estradiol	0.1 nM	n.p.	3 nM
Specific activity of labelled E_2	140 - 150 Ci/mmol	n.p.	n.p.
Test chemical solvent	DMSO	n.p.	n.p.
Concentration range of competing ligand	5, 1, 0.5 µM	n.p.	n.p.
No. of replicates	quadruplicate	n.p.	n.p.
Time of incubation	3 hours	n.p.	1 hour
Temperature of incubation	37ºC	n.p.	37ºC
Measure of nonspecific binding	n.p.	n.p.	n.p.
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	ethanol in PBS	charcoal/dextran in Tris-EDTA, pH 7.4	ethanol
Incubation time and temperature	n.p.	n.p.	n.p.
Centrifugation speed	n.p.	1000g	n.p.
Centrifugation time and temperature	n.p.	10 min, 4ºC	n.p.
Resuspension volume and buffer for pellet	200 µL	n.p.	n.p.
Extraction of label	ethanol	n.p.	ethanol
Data calculations			
Program or method used to calculate data	SigmaPlot	n.p.	n.p.
Data plotted as	linear regression	n.p.	n.p.
Data format in paper (e.g., IC_{50}, K_i)	% displacement of E_2	n.p.	n.p.
Calculation of RBA	from IC_{50}	Scatchard plot	Scatchard plot

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using MCF-7 Cells and Cytosol

Reference	Dodge et al. (1996)	Kramer et al. (1997)	Lascombe et al. (2000)
Preparation of receptor			
Species or cell line	MCF-7 cells	MCF-7 cells	MCF-7 cells
Whole cells/cell homogenate	cell lysate	cytosol	whole cells
Serum source	10% fetal bovine serum	10% fetal bovine serum	0.1% bovine serum
Serum stripping method	dextran/charcoal	dextran/charcoal	dextran/charcoal
Residual E_2 in serum	n.p.	5 pg/ml (18 pm)	n.p.
No. of treated cells	n.p.	n.p.	monolayer culture
Buffer for preparation of cell homogenate or cytosol	n.p.	Tris-EDTA-DTT-molybdate, pH 7.5; 4ºC	n.p.
Protein concentration of cytosol	0.5 mg/ml	n.p.	n.p.
Competitive binding assay			
Volume and concentration of ^{3}H-estradiol	0.5 nM	10 nM	0.1 nM
Specific activity of labelled E_2	n.p.	n.p.	n.p.
Test chemical solvent	n.p.	ethanol	ethanol
Concentration range of competing ligand	0.00001 - 1 µM	70 - 0.01 µM	n.p.
No. of replicates	n.p.	duplicate	quadruplicate
Time of incubation	18 hours	2 hours	1 hour
Temperature of incubation	4ºC	4ºC	37ºC
Measure of nonspecific binding	n.p.	n.p.	n.p.
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	charcoal/dextran; 0.07 ml	hydroxyapatite	ethanol in phosphate buffer, pH 7.4
Incubation time and temperature	n.p.	n.p.	n.p.
Centrifugation speed	n.p.	800g	n.p.
Centrifugation time and temperature	n.p.	10 min, 4ºC	n.p.
Resuspension volume and buffer for pellet	n.p.	n.p.	n.p.
Extraction of label	n.p.	n.p.	ethanol
Data calculations			
Program or method used to calculate data	n.p.	n.p.	Student's t-test
Data plotted as	n.p.	nonlinear regression	% control vs. molar excess of competitor
Data format in paper (e.g., IC_{50}, K_i)	DPM/nM	log IC_{50}	bound E_2 vs molar excess
Calculation of RBA	from IC_{50}	Scatchard plot	% control

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using MCF-7 Cells and Cytosol

Reference	Miodini et al. (1999)	Nagel et al. (1997)	Palomino et al. (1994)
Preparation of receptor			
Species or cell line	MCF-7 cells	MCF-7 cells	MCF-7 cells
Whole cells/cell homogenate	homogenate	whole cells	cytosol
Serum source	2% fetal calf	calf serum	n.p.
Serum stripping method	n.p.	charcoal	n.p.
Residual E_2 in serum	n.p.	n.p.	n.p.
No. of treated cells	n.p.	n.p.	n.p.
Buffer for preparation of cell homogenate or cytosol	K_2HPO_4-EDTA, glycerol, thioglycerol, pH 7.4	n.p.	n.p.
Protein concentration of cytosol	n.p.	n.p.	n.p.
Competitive binding assay			
Volume and concentration of ^{3}H-estradiol	5 nM (16α-I-estradiol)	1 nM	1.5 nM
Specific activity of labelled E_2	8150 GBq/mM	104 Ci/mol	100 Ci/mmol
Test chemical solvent	n.p.	ethanol	n.p.
Concentration range of competing ligand	0.0025 - 25 µM	0.1 - 100 µM	1.5 - 3,000 nM
No. of replicates	quadruplicate	n.p.	triplicate
Time of incubation	overnight	18 hours	overnight
Temperature of incubation	4ºC	37ºC	4ºC
Measure of nonspecific binding	n.p.	n.p.	n.p.
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	n.p.	HBSS/BSA	charcoal/dextran
Incubation time and temperature	n.p.	n.p.	n.p.
Centrifugation speed	n.p.	n.p.	n.p.
Centrifugation time and temperature	n.p.	n.p.	n.p.
Resuspension volume and buffer for pellet	n.p.	1 mL	n.p.
Extraction of label	n.p.	HBSS	n.p.
Data calculations			
Program or method used to calculate data	Latin Square	n.p.	n.p.
Data plotted as	n.p.	n.p.	n.p.
Data format in paper (*e.g.*, IC_{50}, K_i)	% binding	% inhibition vs M	n.p.
Calculation of RBA	n.p.	RBA	Scatchard plot

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using MCF-7 Cells and Cytosol

Reference	Rijks et al. (1996)	Soto et al. (1995)	Stoessel and Leclercq (1986)
Preparation of receptor			
Species or cell line	MCF-7 cells	MCF-7 cells	MCF-7 cells
Whole cells/cell homogenate	homogenate	cytosol	whole cells
Serum source	n.p.	plasma-derived human	fetal calf serum
Serum stripping method	n.p.	dextran/charcoal	
Residual E_2 in serum	n.p.	<0.01 pg/ml	
No. of treated cells		n.p.	monolayer culture
Buffer for preparation of cell homogenate or cytosol	Tris-EDTA-DTT-molybdate, pH 7.4; 4ºC	KCl-EDTA-Tris, pH 7.4	n.p.
Protein concentration of cytosol	1.4 mg/ml	n.p.	n.p.
Competitive binding assay			
Volume and concentration of ^{3}H-estradiol	$4.8x10^{-9}$	2 nM	1 nM
Specific activity of labelled E_2	4.26 TBq/mmol	n.p.	100 Ci/mmol
Test chemical solvent	n.p.	DMSO or ethanol	ethanol
Concentration range of competing ligand	$1x10^{-11}$ - $2x10^{-6}$	1 pM - 1 mM	0.1 nM - 10 µM
No. of replicates	duplicate	n.p.	triplicate
Time of incubation	18 hours	16 hours	50 min
Temperature of incubation	0 - 4ºC	4ºC	37ºC
Measure of nonspecific binding	n.p.	n.p.	n.p.
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	charcoal/dextran in SHBG	charcoal/dextran	n.p.
Incubation time and temperature	4ºC	n.p.	n.p.
Centrifugation speed	800g	n.p.	n.p.
Centrifugation time and temperature	7 min; 4ºC	n.p.	n.p.
Resuspension volume and buffer for pellet	n.p.	n.p.	n.p.
Extraction of label	n.p.	n.p.	n.p.
Data calculations			
Program or method used to calculate data	n.p.	n.p.	n.p.
Data plotted as	n.p.	n.p.	n.p.
Data format in paper (e.g., IC_{50}, K_i)	n.p.	n.p.	RBA
Calculation of RBA	n.p.	n.p.	IC_{50} E_2/IC_{50} test compound X 100

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using MCF-7 Cells and Cytosol

Reference	VanderKuur et al. (1993)
Preparation of receptor	
Species or cell line	MCF-7 cells
Whole cells/cell homogenate	whole cells
Serum source	5% calf serum
Serum stripping method	none
Residual E_2 in serum	none
No. of treated cells	n.p.
Buffer for preparation of cell homogenate or cytosol	n.p.
Protein concentration of cytosol	n.p.
Competitive binding assay	
Volume and concentration of ^{3}H-estradiol	n.p.
Specific activity of labelled E_2	n.p.
Test chemical solvent	n.p.
Concentration range of competing ligand	n.p.
No. of replicates	n.p.
Time of incubation	n.p.
Temperature of incubation	4ºC
Measure of nonspecific binding	n.p.
Separation of ligand	
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	charcoal/dextran
Incubation time and temperature	n.p.
Centrifugation speed	n.p.
Centrifugation time and temperature	n.p.
Resuspension volume and buffer for pellet	n.p.
Extraction of label	n.p.
Data calculations	
Program or method used to calculate data	n.p.
Data plotted as	n.p.
Data format in paper (e.g., IC_{50}, K_i)	n.p.
Calculation of RBA	Scatchard plot

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

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Appendix B

In Vitro ER Binding Assay Protocols

B1 Protocol for the Competitive ER binding MCF-7 (Whole Cell Assay)
(Provided by Dr. Guy Leclercq, Clinique et Laboratoire de Cancerologie Mammaire, Centre des Tumeurs de l'Universite Libre de Bruxelles, Brussels, Belgium)

B2 Protocol for the Fluorescence Polarization Assay of the Competitive Binding of Ligands to Estrogen-Receptor Complexes
(Provided by Dr. William Allworth, Department of Chemistry, University of New Orleans, New Orleans, LA, USA)

B3 Protocol for the Competitive Ligand Binding Assay
(Provided by Dr. Timothy Zacharewski, Dept. of Biochemistry, Michigan State University, Lansing, MI, USA)

B4 Standard Operating Procedure for the Rat Estrogen Receptor Equilibrium Exchange Assay
(Provided by Dr. Weida Tong, Division of Genetic and Reproductive Toxicology, National Center for Toxicological Research, Jefferson, AR, USA)

B5 Protocol for the Estrogen Receptor Competitive Binding Assay Using Rat Uterine Cytosol
(Provided by Dr. Susan Laws, U.S. EPA, NHEERL, Research Triangle Park, NC, USA and Mr. Gary Timm, U.S. EPA, Washington, DC, USA)

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Appendix B1

Protocol for the Competitive ER binding MCF-7 (Whole Cell Assay)

(Provided by Dr. Guy Leclercq, Clinique et Laboratoire de Cancerologie Mammaire, Centre des Tumeurs de l'Universite Libre de Bruxelles, Brussels, Belgium)

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Stoessel and Leclercq, J Steroid Biochemistry **25**(5A):677-682, 1986.

PROTOCOL FOR THE COMPETITIVE ER BINDING

(Whole Cell Assay)

1. Culture conditions: MCF-7 cells are maintained at 37°C in a 5% CO_2 atmosphere in phenol red-free minimal essential medium (MEM) containing 10% charcoal stripped calf serum and penicillin, streptomycin, glutamine.

2. MCF-7 cells (20,000 cells/ml) are incubated for 4 days in 24 multiwells (NUNC) under above mentioned conditions.

3. After 4 days of culture, the medium is removed and the cells are incubated for 1 hour at 37° C with 1 nM [^{3}H]estradiol (E_2) or investigated compound (X) at concentrations ranging from 1 nM to 1µM.

4. Medium is again removed and the cells washed twice with phosphate buffer saline (PBS).

5. 250 µl absolute ethanol are added to each well (exposition during 20 min).

6. Aliquots of 200 µl of supernatant (ethanol extract) are added to 3.8 ml of scintillation liquid for radioactivity measurements (10 min, counting).

7. RBA data are established from the mean of 3 independent experiments, each performed in triplicate.

8. Relative binding affinity: $RBA = \dfrac{(I_{50})E_2}{(I_{50})\ x} \times 100$

   I_{50} = concentration producing 50% inhibition of [^{3}H]E_2 incorporation (dpm of ethanol extracts).

Brussels, December 2001.

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Appendix B2

Protocol for the Fluorescence Polarization Assay of the Competitive Binding of Ligands to Estrogen-Receptor Complexes

(Provided by Dr. William Allworth, Department of Chemistry, University of New Orleans, New Orleans, LA, USA)

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Fluorescence Polarization Assay of the Competitive Binding of Ligands to Estrogen-Receptor Complexes

Purified expressed human estrogen receptor alpha (ER_α) and human estrogen receptor beta (ER_β), fluorescent-labeled 17β-estradiol (ES2), and estrogen screening buffer were purchased from Pan Vera Corporation.

A solution of ER-ES2 complex in estrogen screening buffer containing 20 nM ES2, 26 nM human-ER and 10 mM dithiothreitol (DTT) was prepared, and 50 µ1 of this solution was added to borosilicate test tubes containing 50 µ1 of serially diluted compounds to be tested as potential estrogenic ligands. Samples tubes containing 50 µl of the estrogen screening buffer and 50 µ1 of the ER-ES2 complex solution were included as negative controls to determine the initial polarization value (P_o, polarization value with no estrogen competitor present). Sample tubes containing 100 µ1 of the estrogen screening buffer with 10 nM ES2 and 5 mM DTT were also included to determine the polarization value of nonbound ES2 (P_{100}). The samples were incubated at room temperature for 1 hour and the fluorescence polarization (FP) then measured using a Beacon 2000 Fluorescence Polarization Instrument (Pan Vera) equipped with a 530 nm emission filter and a 490 nm excitation filter.

FP values were converted to percentage inhibition using the equation

$$I_{\%} = (P_o - P)/(P_o - P_{100}) \times 100$$

where P_0 is the FP value at 0%, P_{100} is the FP value when 100% of the ES2 has been competitively displaced from the ER complex, and P is the experimental FP value at each concentration of the competing ligand being tested. The percentage inhibition versus competitor concentration curves were analyzed by nonlinear least-squares curve fitting and the concentration of competing ligand required to displace half of the bound ES2 fluorescent ligand determined (IC_{50}). The IC_{50} values were converted to relative binding affinities (RBA) using 17β-estradiol (E_2) as a standard. The RBA for E_2 was set equal to 100 and the RBA value for each competing ligand calculated using the following formula:

$$RBA = (IC_{50}E_2 / IC_{50}\ \text{competitor}) \times 100\ .$$

Measuring Estrogen Receptor-Estrogen Response Element Binding by Fluorescence Polarization

Preparation of fluorescence labeled estrogen response elements.

Sense and antisense oligonucleotide strands 35 bases long containing either estrogen response elements (EREs) from the Xenopus vit A2 gene or the human pS2 gene, or the consensus glucocorticoid response element (negative control) were obtained from Oligos Etc. (Wilsonville, OR). The sense DNA strands were labeled with fluorescein attached via a six-carbon spacer at the 5' terminus. Double stranded oligonucleotides were then prepared by annealing equimolar concentrations of the separate sense and antisense strands in 10 mM Tris-HCl, pH 7.8, and 1.50

mM NaCl as follows: 1 nmole of the sense and 1 nmole of the antisense DNA strands in 500 µL buffer were heated in water bath to 95°C for 10 min and slowly cooled (30 min) to room temperature. To remove any hairpin formations the double stranded DNA was purified by electrophoresis on 12% polyacrylamide (1:19 bisacrylamide:acrylamide) gels containing 89 mM Tris-borate, 2.5 mM EDTA, pH 8.3, and 10% ammonium persulphate.

ER-ERE binding studies.

The abilities of ligand bound ERα and ERβ to associate with Xenopus vit A2 ERE or human pS2 ERE were measured using fluorescence polarization (FP). Purified, expressed human ERα and ERβ obtained from Pan Vera Corporation were serially diluted from 450 nM to 0.8 nM in DNA binding buffer (10 mM potassium phosphate, pH7.8; 0.1 mM EDTA; 50 µM magnesium chloride; 10% glycerol). The concentrations of the ligands required to saturate ERα or ERβ were determined by FP competitive binding experiments. Each ER was then incubated with saturating levels of the individual estrogenic ligands for 30 min, and then for 10 min with poly (dI-dC) (1 µg/5 µg of protein) at room temperature. The binding, initiated by adding fluorescein-labeled synthetic oligonucleotide EREs (final concentration 0.5 nM), was allowed to proceed at room temperature for 60 min in dark. The same experiment was performed with the ERs bound to 17β-estradiol (E_2) as positive controls. The samples were prepared and measured in borosilicate test tubes with final reaction volume of 100 µl. The FP at each ER concentration was measured on Beacon 2000 Fluorescence Polarization Instrument (Pan Vera Corporation) equipped with 490 nm excitation and 530 nm emission filters. Binding isotherms were constructed by plotting percent saturation versus ER concentration using the formula:

$$S_{\%} = (P-P_0)/(P_{100}-P_0) \times 100$$

where P_0 is the polarization value at 0% saturation, P_{100} is the polarization value at 100% saturation, and P is the observed fluorescence polarization (FP) at each concentration point. The equilibrium dissociation constant (K_d) was calculated from the binding curves using a nonlinear least-squares curve fitting program. To compare the binding affinities of ERα-ligand and ERβ-ligand complexes for the various EREs the K_d values were converted to relative binding affinities (RBA) using the following formula with the K_d for the E_2-ER complex as the standard.

$$RBA = (K_d E_2 / K_d \text{ competitor}) \times 100.$$

Appendix B3

Protocol for the Competitive Ligand Binding Assay

(Provided by Dr. Timothy Zacharewski, Dept. of Biochemistry, Michigan State University, Lansing, MI, USA)

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Competitive Ligand Binding Assay

1. Caution: this protocol requires the use of radioactivity. Proper handling and disposal of all radioactive samples should be followed as outlined by the institution's safety office.
2. Prepare TEGD buffer by adding DTT to a final concentration of 1 mM to TEG buffer.
3. Add BSA (carrier protein) to a final concentration of 1 mg/ml.
4. Thaw receptor on ice. Using the appropriate dilution factor, add the receptor. As a rule of thumb, ~25 ml of TEGD+receptor is required per 96-well plate (240 µl per tube). Keep on ice until adding to tubes in step 7.
5. Label rack of 96 1 ml glass test tubes (Marsh Scientific).
6. Add 5 µl of radiolabeled compound at appropriate concentration to each tube using 8-channel pipettor.
7. Pipet 5 µl of unlabeled competitor into each tube. A typical assay may involve 5 concentrations of competitor plus solvent alone, with each concentration being run in quadruplicate. For example: A1-D1 are DMSO, A2-D2 have compound A at 10^{-10}M, A3-D3 have compound A at 10^{-9}M, ... and A6-D6 have compound A at 10^{-6}M. A similar scheme is set up for compound B (A7-D12), compound C (E1-H6), and compound D (E7-H12). Typically, compound A is the 'cold' version of the radiolabeled compound.
8. Transfer 240 µl of TEGD+receptor to each tube, using 8-channel pipettor.
9. Label and place an empty test tube rack on ice. Vortex each test tube from step 8 and place in new rack, ensuring that the order of tubes remains unchanged.
10. Place cover on glass tube rack and incubate at 4°C for 24 hrs.
11. At time of harvest, fill head of harvester with millipore-filtered water and place the head of the harvester (Packard Filtermate 196) into position.
12. Put the positioning bracket in place. Invert the 'wash' filter place and place it within the bracket so position A12 is now in the top left. Gently close the harvester unit by pulling down the lever.
13. Turn the pump on.
14. Push and lock the 'cold' vacuum circuit on the harvester. Placing the collection tray against the intake vacuum inlets and wash for 30 sec.
15. Dry the wash plate by opening the harvester and applying continued suction for 10 sec.
16. Place a new filter plate in the harvester. Rinse the plate once with 50 ml of cold TEG buffer using the 'cold' circuit.
17. Switch to 'hot' circuit. Place the rack of 96 test tubes underneath the head unit and raise into place until all the liquid has passed through the harvester.
18. Wash with 3 x 50 ml of cold TEG buffer, leaving harvester on 'hot' circuit for all three washes. Dry as in step 15.
19. Wash harvester as in steps 11-14.
20. Label and date the filter plate. Place in radioactive hood for 10 min.
21. Put back seal on each plate and add 50 µl of Microscint20 (Packard) to each well. Remember that the plate is now inverted (tube A1 is now bound to filter location A12).
22. Put top seal on plate and incubate at room temperature at least 30 min. Count the plate using the Receptor Binding Protocol on the TopCount Scintillation counter (Packard).

TEG Buffer

10 mM Tris
1.5 mM EDTA
10% Glycerol
pH 7.6

GST Purification

Bacterial overexpression

1. Transform *E. coli* BL 21 cells with appropriate pGEX vector. Allow colonies to grow all day.
2. Pick 2-3 colonies at the end of the day and allow them to grow overnight in 3 ml LB-Amp.
3. Perform miniprep. Add more LB-Amp (~2 ml) to starter cultures and place in shaker incubator all day (6-8 hr) at 37°C and 225 rpm.
4. Check miniprep using appropriate restriction enzymes. Select a single colony for overexpression.
5. Inoculate 50 ml of LB-Amp with 500 µl of starter culture. Incubate overnight at 37°C and 225rpm.
6. Inoculate 500 ml of LB-Amp with 5 ml of culture. (Often ~6L (i.e. 12 flasks) are inoculated.) Grow at 37°C and 225 rpm.
7. Induce culture with 0.5 mM IPTG (final conc.) when culture reaches $O.D._{600}$ of 1.0 (~3.5 hr). Grow induced culture for 3.5 hr at 37°C.
8. Pellet 500 ml culture by centrifuging 10 min at 5000 rpm using a Beckman JA-14 rotor. Remove supernatant, and a second 500 ml culture can be added to the same tube and centrifuged as above.
9. Remove supernatant and store at –80°C.

Fusion protein extract – should be done at 4°C or on ice

10. Resuspend pellet (from the equivalent of 1L of culture) in 25 ml of resuspension buffer containing protease inhibitors and 5 mM DTT added fresh. Transfer resuspended cells into 50 ml centrifuge tube.
11. Disrupt cells by sonication. Keep tube on ice at all times. Use the pulse mode at setting 3 and sonicate cells for 3x20 sec.
12. Add Tween20 to a final concentration of 0.3%. Incubate at 4°C under constant shaking for 30-60 min.
13. Centrifuge at 20,000g (15,000rpm using the SS-34 rotor) for 30 min at 4°C.
14. Filter supernatant to eliminate cellular debris that did not pellet.
15. Transfer supernatant to a 50 ml tube.

Protein purification – degas all buffers and GSH matrix before setting up the column

16. Add 10 ml of packed matrix to 20 ml glass column. Place the adaptor at an appropriate distance from the top of the matrix in order to reduce the void volume. Hook the column up to the peristaltic pump in the following order: buffer, pump, followed by the column.
17. Be sure that there are NO air bubbles in the lines or the column.
18. Equilibrate the matrix with 5x bed volume (50 ml of equilibration buffer). Keep the flow rate at 0.5 ml/min. Steps 15-17 should be done in advance to allow the column to properly equilibrate.
19. Stop the pump between buffer transfers and wait briefly before transferring the collection line to a different buffer.
20. Place the collection line into the 50 ml tube containing the crude protein. (A 250 µl aliquot of the crude sample should be saved for subsequent analysis.) Keep flow rate at 0.5ml/min.
21. Collect the flowthrough and save a sample for subsequent analysis.
22. Wash the column with 10x bed volume (80-100 ml) of wash buffer.
23. Elute fusion protein with 2x bed volume (20 ml) of elution buffer.
24. Collect eluate in a 50 ml tube.
25. Concentrate protein to 0.5 mg/ml using the Amicon 50,000 MWCO spin column.
26. Check protein concentration using the Bradford method.

Equilibration/Resuspension Buffer

50 mM HEPES
3 mM EDTA
50 mM NaCl
10% glycerol
pH 7.5

Add prior to use (final conc.)
10 ug/ml Pepstatin A (from 1 mg/ml stock in ethanol)
10 ug/ml Leupeptin (from 1 mg/ml stock in water)
100 ug/ml PMSF (from 10 mg/ml stock in isopropanol)
5 mM DTT (from 1M stock in water)

Column Wash Buffer

50 mM HEPES
3 mM EDTA
150 mM NaCl
10% glycerol
pH 7.5

Elution Buffer

50 mM HEPES
3 mM EDTA
150 mM NaCl
10% glycerol
10 mM glutathione
pH 8.0

Appendix B4

Standard Operating Procedure for the Rat Estrogen Receptor Equilibrium Exchange Assay

(Provided by Dr. Weida Tong, Division of Genetic and Reproductive Toxicology, National Center for Toxicological Research, Jefferson, AR, USA)

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Rat Estrogen Receptor Equilibrium Exchange Assay

Standard Operating Procedure

1.0 Purpose & Applicability

The purpose of this SOP is to outline a procedure for the quantitation of estrogen receptor number and binding affinity in ovariectomized adult female rat reproductive tissue (i.e., uterus). As tissue receptor number is finite, the binding of ligand to the receptor (i.e., specific binding) is a saturable process. Unsaturable binding of ligand is called nonspecific binding and is due to ligand binding to non-receptor proteins, etc. Total binding is saturable binding + unsaturable binding. Total and nonspecific binding are determined empirically, while specific binding is calculated as their difference (i.e., total - nonspecific). The assay described below measures the binding of radiolabeled synthetic ligand (i.e., [^{3}H]-Estradiol) by cytosolic and/or nuclear receptor extracts. Total [^{3}H]-Estradiol binding is determined by incubating the extracts with increasing concentrations of [^{3}H]-Estradiol during which time the labeled ligand binds to the unoccupied receptors in the cytosol extract or exchanges with endogenous hormone bound to the nuclear receptors. The total bound ligand (i.e., saturable + nonsaturable binding) is separated from free ligand via hydroxylapatite extraction, eluted from the receptor with ethanol and quantified using liquid scintillation counting. Nonspecific binding is determined exactly as above except that a 100-300 fold molar excess of radioinert estradiol is included in each incubation together with the increasing concentrations of [^{3}H]-Estradiol (i.e., binding of [^{3}H]-Estradiol in the presence of a 100-300 fold molar excess of radioinert estradiol represents unsaturable binding). Specific binding is calculated as total - nonspecific binding and is analyzed via Scatchard analysis.

2.0 Safety and Operating Precautions

All procedures with radioisotopes should follow the regulations and procedures as described in the Hazardous Agent Protocol (HAP) and in the Radiation Safety Manual and Protocols.

3.0 Equipment and Materials

3.1 Equipment

* Corning Stir/Hot Plates
* Digital Pipets
* Balance
* Polytron PT 35/10 Tissue Homogenizer
* Beckman HPLC with on-line Radiochromatograph
* Vacuum Concentrator
* Hamilton Syringes (50 µl)
* Refrigerated General Laboratory Centrifuge
* Beckman LX Ultracentrifuge with 90 TI Rotor
* pH Meter with Tris-compatible electrode
* Scintillation counter

3.0 Equipment and Materials (cont.)

3.2 Chemicals

* Tris HCL
* Tris Base
* Glycerol (99% +)
* Ethylenediaminetetraacetic Acid (EDTA); Disodium Salt
* Dithiothreitol (DTT)
* Hydroxylapatite (Bio-Rad)
* Scintillation Cocktail
* Ethyl Alcohol, anhydrous
* [^{3}H]-Estradiol
* Radioinert Estradiol (Steraloids; recrystallized)
* Steroids (Steraloids; recrystallized)

3.3 Supplies

* 20 ml Polypropylene Scintillation vials
* 12x75 mm Borosilicate glass test tubes
* 1000 ml graduated cylinders
* 500 ml erlenmeyer flasks
* yellow (0-200 µl) pipet tips

4.0 Methods

4.1 Preparation of TEDG Stock Solutions

(A) 200 mM EDTA

Add 7.444 g EDTA (disodium salt) to 100 ml ddH_2O. Store at 4^{o}C

Use 750 µl/100 ml TEDG buffer = 1.5 mM final concentration of EDTA

(B) 1.0 M Tris

Add in a volumetric Flask:
147.24 g Tris HCL
8.0 g Tris Base
800 ml ddH2O
Stir until dissolved. QS to 1.0 Liter. Refrigerate to 4^{o}C and then pH the cooled solution to 7.4. pH using standardizing solutions which are also at 4^{o}C. Store at 4^{o}C. Use 1.0 ml of 1.0 M Tris/100 ml TEDG buffer = 10 mM final Tris concentration.

4.2 Preparation of TEDG Buffer (pH 7.4)

Add the following in this order:

Ingredient	To make 100 ml	To make 500 ml	To make 1.0 L	To make 2.0 L
dd H2O	87.15 ml	435.75 ml	871.5 ml	1743.0 ml
1.0 M Tris	1.00 ml	5.00 ml	10.0 ml	20.0 ml
Glycerol	10.00 ml	50.00 ml	100.0 ml	200.0 ml
200 mM EDTA	750 µl	3.75 ml	7.5 ml	15.0 ml

Immediately prior to use in the assay, add:

15.4 mg Dithiothreitol/100 ml TEDG buffer. pH final solution to make sure it is 7.4 at 4 ^{o}C

4.3 Preparation of 50 mM Tris Buffer

Ingredient	To make 1.0 L	To make 2.0 L	To make 5.0 L
1.0 M Tris	50 ml	100 ml	250 ml
ddH_2O	950 ml	1900 ml	4750 ml

Store at 4 ^{o}C. pH final solution to make sure it is 7.4 at 4^{o}C

4.4 Preparation of 60% Hydroxylapatite (HAP) Slurry

Shake Bio-Rad HT-GEL until all the HAP is in suspension (i.e., looks like milk). The evening before the receptor extraction, pour 100 ml of the suspension into a 100 ml graduated cylinder, parafilm seal the top and place in the refrigerator for at least 2 hours. Pour off the phosphate buffer supernatant and bring the volume up to 100 ml with 50 mM Tris buffer. Resuspend the HAP by sealing the top of the graduated cylinder with parafilm and inverting the cylinder several times. Place in the refrigerator overnight. The next morning, repeat the washing steps twice more with fresh 50 mM Tris buffer. After the last wash, add enough 50 mM Tris buffer to make the final solution a 60% slurry (i.e., if the volume of settled HAP is 60 ml, bring the final volume of the slurry up to 100 ml). Store at 4^{o}C until ready for use in the extraction.

4.5 Preparation of [^{3}H]-Estradiol Stock Solutions

Dilute the original 1.0 mCi/ml stock of [^{3}H]-Estradiol to 0.1 µM (i.e., 1 x 10^{-7} M). This is most easily accomplished by pipeting 1 µl of the stock solution for every specific activity unit (Ci/mmol) and diluting this to 10.0 ml with ethanol. Thus, if the specific activity of the stock vial = 86 Ci/mmol, then pipet 86.0 µl into an amber colored vial and add 10.0 ml ethanol to the vial; this solution is 1 x 10^{-7} M.

Calculation Check:
86 µl x 1.0 mCi/1000 µl = 86 x 10^{-3} mCi Estradiol = 86 x 10^{-6} Ci Estradiol
86 x 10^{-6} Ci ÷ 86.0 Ci/mmol = 1 x 10^{-6} mmol Estradiol = 1 x 10^{-9} moles Estradiol
1 x 10^{-9} moles Estradiol ÷ .010 liters = 1 x 10^{-7} moles/liter = 0.1 µM

To prepare the 1 x 10^{-8} M stock, simply make a 10-fold dilution of the 1 x 10^{-7} M stock. To do this, pipet 1.0 ml of the 1 x 10^{-7} stock into a clean amber colored vial and add 9 ml ethanol. Final concentrations = 0.01 µM.

4.6 Preparation of 100X Radioinert Estradiol Solutions

Add 27.24 mg Estradiol to a 100 ml volumetric flask, QS to 100 ml with 95% ethanol (1 x 10^{-3} M or 1 mM stock).

Take 1.0 ml of the 1 mM stock estradiol and place in another 100 ml volumetric flask, QS new flask to 100 ml with 95% ethanol (1 x 10^{-5} or 10 µM). This is the 10 µM radioinert estradiol stock and should be stored in the freezer when not in use (storage in 20 ml aliquots works well).

To make the **1.0 µM radioinert estradiol stock**: Pipet 2.0 ml of the 10 µM estradiol stock into a vial and dilute to 20 ml with 95% ethanol (1 x 10^{-6}).

To make the **0.1 µM radioinert estradiol stock**: Pipet 2.0 ml of the 1.0 µM estradiol stock into a vial and dilute to 20 ml with 95% ethanol (1 x 10^{-7}).

4.7 Standard Curve Construction for Saturation and Scatchard Analysis

The first step is to pipet the radioactive ligand (i.e., [^{3}H]-Estradiol) with and without a 100-fold excess of radioinert estradiol into each tube so that the final concentrations of [^{3}H]-Estradiol are 7.0, 3.5, 1.17, 0.7, 0.35, 0.117, 0.035, and 0.0117 nM in a 300 µl total volume. To accomplish this, label tubes and pipet the following into duplicate 12x75 mm borosilicate glass test tubes:

Tube	Volume[^{3}H]-E_2 (µl)	Final Conc. [^{3}H]-E_2 (nM)	Volume Radioinert E_2 (µl)	Final Conc. Radioinert E_2 (nM)	Volume of50 mM Tris (µl)	Cytosolic Extract (µl)
1	**21 of 1x10^{-7}**	7.00	*****	*****	**229**	**50**
2	**10.5 of 1x10^{-7}**	3.50	*****	*****	**239.5**	**50**
3	**3.5 of 1x10^{-7}**	1.17	*****	*****	**246.5**	**50**
4	**21 of 1x10^{-8}**	0.70	*****	*****	**229**	**50**
5	**10.5 of 1x10^{-8}**	0.35	*****	*****	**239.5**	**50**
6	**3.5 of 1x10^{-8}**	0.117	*****	*****	**246.5**	**50**
7	**10.5 of 1x10^{-9}**	0.035	*****	*****	**239.5**	**50**
8	**3.5 of 1x10^{-9}**	0.0117	*****	*****	**246.5**	**50**
9	**21 of 1x10^{-7}**	7.00	**21 of 1x10^{-5}**	700	**208**	**50**
10	**10.5 of 1x10^{-7}**	3.50	**10.5 of 1x10^{-5}**	350	**229**	**50**
11	**3.5 of 1x10^{-7}**	1.17	**3.5 of 1x10^{-5}**	117	**243**	**50**
12	**21 of 1x10^{-8}**	0.70	**21 of 1x10^{-6}**	70	**208**	**50**
13	**10.5 of 1x10^{-8}**	0.35	**10.5 of 1x10^{-6}**	35	**229**	**50**
14	**3.5 of 1x10^{-8}**	0.117	**3.5 of 1x10^{-6}**	11.7	**243**	**50**
15	**10.5 of 1x10^{-9}**	0.035	**10.5 of 1x10^{-7}**	3.5	**229**	**50**
16	**3.5 of 1x10^{-9}**	0.0117	**3.5 of 1x10^{-7}**	1.17	**243**	**50**

After all ingredients have been pipeted, gently vortex the incubation tubes, place them in the tube rotator at 4°C and incubate for approximately 20 hours. Set rotator speed at approximately 40%. Proceed to **Day 2** of assay instructions.

Note: tubes #1-8 are Total Binding Tubes and tubes #9-16 are Non-specific Binding Tubes

4.8 Estrogen Receptor Assay Procedure (Keep everything at 4°C!!!)

1. Estrogen Receptor Preparation:
   a) Make TEDG buffer (add the DTT and check pH) and place in ice.
   b) Ovariectomize 10-12 Sprague-Dawley rats at least 10 days prior to receptor preparation.
   c) Sacrifice the rats and remove the uterus from each animal. Trim fat from the uteri.
   d) Weigh each uteri and record the data.
   e) Place all the uteri into a homogenization tube containing TEDG buffer at 4°C.
   f) Decant storage buffer from uteri and add 1.0 ml TEDG buffer per 0.1 gm of tissue.
   g) Homogenize the tissue at 4°C with a Polytron homogenizer using 5-sec bursts. Note: Polytron should be cooled prior to use by placing the probe in TEDG buffer in an ice water bath.
   h) Transfer the homogenate to pre-cooled ultracentrifuge tubes, balance and centrifuge at 105,000 x *g* (approximately 33,000 rpm with TI-90 Beckman ultracentrifuge rotor) for 60 min. at 4°C.
   i) The supernatant contains the cytosolic estrogen receptors. Decant supernatant and assay directly or freeze (-70°C) until ready for use.

Day 1

Note: If constructing a standard curve for saturation and Scatchard analysis, label tubes and pipet reagents as described in section 4.7. If running a competitive binding assay, start at step 3 and proceed from there.

2. Label **duplicate** 12x75 glass tubes.
   a) *Standard Curve*: label tubes 0, NSB, S1, S2, S3, S4, S5

Standard Label	Initial Conc. (M)	Final Conc. (M)
0	0	0
NSB	3×10^{-6}	1×10^{-7}
S1	3×10^{-7}	1×10^{-8}
S2	3×10^{-8}	1×10^{-9}
S3	1×10^{-8}	3.33×10^{-10}
S4	3×10^{-9}	1×10^{-10}
S5	1×10^{-9}	3.33×10^{-11}

b) *Test Chemicals*: Label tubes 1, 2, 3, 4, ... The concentrations tested can vary, but a potential standard range of concentrations is outlined below. More than one chemical can be run in an assay if desired.

Sample #	Initial Conc (M)	Final Conc. (M)
1	3×10^{-2}	1×10^{-3}
2	3×10^{-3}	1×10^{-4}
3	3×10^{-4}	1×10^{-5}
4	3×10^{-5}	1×10^{-6}
5	3×10^{-6}	1×10^{-7}
6	3×10^{-7}	1×10^{-8}

3. Pipet 10 µl of [^{3}H]-estradiol (initial conc. = 3×10^{-8} M; final conc. = 1×10^{-9} M) into all tubes.
4. Pipet 10 µl of estradiol standard to appropriate standard tubes. The 0 tube receives 10 µl of ethanol.
5. Pipet 10 µl of radioinert test chemical to appropriate sample tubes.
6. Pipet 230 µl of 50 mM Tris buffer into each tube.
7. Pipet 50 µl of cytosolic estrogen receptor supernatant to all tubes.
8. Place reaction mixture tubes in rotator at 4°C for 20 hours.
9. Before leaving for the day, prepare the first wash of the HAP slurry as described in Section 4.4.

Day 2

10. Finish washing the HAP as described in Section 4.4. Dilute with 50 mM Tris to yield a 60% slurry and transfer contents to a 100 ml Erlenmeyer flask. Place a stir bar in the flask and place the flask into a beaker of ice water. Stir the HAP slurry by placing the beaker on a stir plate.
11. Label duplicate 12x75 glass tubes with standard & sample numbers and place on ice. These are the HAP tubes.
12. While the slurry is constantly being stirred, pipet 500 µl of the HAP slurry into the cold, pre-labeled 12x75 tubes.
13. Remove the reaction mixture tubes from the rotator and place them in the ice water bath with the HAP tubes.
14. After mixing the contents of the reaction mixture tubes, pipet 200 µl of each reaction mixture into the appropriately-labeled, duplicate HAP tubes. *Discard remainder of reaction mixture, unless doing the Saturation & Scatchard Analysis.*
15. Vortex the HAP tubes at 5 minute intervals for a total of 20 minutes. *During this incubation, if you are running the Saturation & Scatchard Analysis, pipet 30 µl of the remaining reaction mixture into duplicate, appropriately-labeled, scintillation vials (these are called the TotalCount Tubes and will be used to estimate the concentration of total [^{3}H]-estradiol).*
16. Centrifuge the HAP tubes at 4°C for 3-4 minutes at 600 x *g* (~1700 rpm).
17. Place the tubes back into the ice water bath and aspirate and discard the supernatant. A vacuum aspiration apparatus is helpful with this step.
18. Add 2.0 ml of 50 mM Tris buffer to each HAP tube. Vortex to resuspend HAP pellet and then centrifuge at 4°C for 3-4 minutes at 1700 rpm.

19. Aspirate and discard the supernatant. Repeat Step 18 twice more.
20. After the third wash, aspirate the supernatant. Add 2.0 ml of cold (4°C) 100% ethanol to each HAP tube. Vortex and place in ice for 15 minutes, vortexing at 5 minute intervals.
21. Centrifuge the HAP tubes at 4°C for 10 minutes at 1700 rpm.
22. Decant the supernatant into appropriately-labeled scintillation vials.
23. Add 10 ml of scintillation cocktail to each vial, cover and shake.
24. Place into scintillation counter and count DPMs.

Evaluation of Data

25. The counts of tube >0' (no radioinert compound added) serve as the comparator for the counts from tubes into which radioinert test compound was added.
26. Determine the percentage of binding: Number of counts from the HAP sample tubes divided by the number of counts from the >0' tube.
27. Plot the percentage of binding as a function of the concentration of the radioinert compound.
28. Determine the IC_{50} by using the biostatistics program KELL or by noting where the binding curve intersects the 50% value of the ordinate.
29. The relative binding affinity (RBA) of each chemical is determined by:

$$RBA = IC_{50}\ Estradiol / IC_{50}\ Test\ Compound$$

5.0 Data Processing

5.1 Free Concentration of [^{3}H]-estradiol

Multiply the DPM in the total count tubes by 1.8047×10^{-5}. **NOTE:** This number will change as the specific activity of new batches of isotope change. Use the equation below to calculate the new number for each batch of isotope.

This value will yield the free concentration (i.e., nM) of [^{3}H]-estradiol initially present in each incubation tube.

Calculation Check

(**X** DPM ÷ 2.22×10^{12} dpm/Ci) = (4.5045×10^{-13} Ci ÷ Specific Activity of [^{3}H]-estradiol Ci/mmole)

= (5.4141×10^{-15} mmole ÷ 1000 mmole/mole) = (5.4141×10^{-18} moles ÷ Volume of reaction mixture)

= (1.8047×10^{-14} moles/liter ÷ 1×10^{-9} moles/nmole) = **X** = 1.8047×10^{-5} nM

Notes: 1. 2.22×10^{12} = a nuclear constant
2. Specific Activity will vary between batches of [^{3}H]-estradiol
3. 1000 mmole/mole is used to convert mmoles to moles

4. Volume of reaction mixture should be in Liters
5. $1x10^{-9}$ moles/nmole is used to convert moles to nmoles

5.2 Calculation of Total, Nonspecific and Specific [^{3}H]-Estradiol Binding

* Total binding = (**X** DPM from the tubes that contained only [^{3}H]-estradiol x $1.6242x10^{-2}$). This value will be total binding in fmoles.

* Nonspecific binding = (**X** DPM from the tubes containing both [^{3}H]-estradiol + 100-fold molar excess radioinert estradiol x $1.6242x10^{-2}$). This value will be nonspecific binding in fmoles.

* Specific binding = (fmoles total binding - fmoles nonspecific binding)

Calculation Check
To get fmoles, multiply the DPM values by $1.6242x10^{-2}$. This is simply nM x 300.

[$1.0847x10^{-5}$ nM x (Volume counted ÷ $1x10^{-6}$ nmoles/fmole)] = $1.6242x10^{-2}$ fmoles

Note: The value $1.6242x10^{-2}$ will change with specific activity of the [^{3}H]-estradiol batch and the volume of the reaction mixture counted.

5.3 Graphical Presentation of the Data

Maximal binding capacity (Bmax) and association/dissociation constants (Ka/Kd) can be estimated using a number of commercially available iterative nonlinear regression analysis programs. One of the better programs was developed by Munson and Rodbard and is called LIGAND (Munson PJ, Rodbard D. Anal. Biochem. 1980; 107:220-239).

6.0 References

Nonneman DJ, Ganjam VK, Welshons WV, Vom Saal FS. Biol. Reprod. 1992; 47:723-729

Segel IH. 1975. Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems. 1st Ed, John Wiley and Sons, Inc., New York, NY.

Korach KS. Endocrinology 1979; 140:1324-1332

Korach KS et al. Molecular Pharmacology 1988; 33:120-126

Kun Chae et al. Molecular Pharmacology 1991; 40:806-811

Appendix B5

Protocol for the Estrogen Receptor Competitive Binding Assay Using Rat Uterine Cytosol

(Provided by Dr. Susan Laws, U.S. EPA, NHEERL, Research Triangle Park, NC, USA and Mr. Gary Timm, U.S. EPA, Washington, DC, USA - 10/22/2001)

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Protocol for the Estrogen Receptor Competitive Binding Assay Using Rat Uterine Cytosol

Purpose of Assay: This assay can be used to determine the relative binding affinities of environmental chemicals for the estrogen receptor (ERα, ERβ) as compared to 17β-estradiol. Data produced using this assay can be used (1) as a screening tool to detect chemicals with possible estrogenic or anti-estrogenic properties; and (2) for development of Quantitative Structure Activity Relationship models to predict the ability of a chemical to bind to the ER.

Distribution of protocol: A final version of this protocol will be distributed as a guide to multiple laboratories, some with previous experience in conducting receptor binding assays, and others with limited or no experience. This protocol is intended to serve as a guide by providing sufficient information to successfully conduct the assay, yet not being totally definitive so that labs already proficient in conducting the assay would be prevented from using well-documented procedures currently in use in their labs.

Terminology:

E2: estradiol, 17β-estradiol, inert estradiol

^{3}H-E2: radiolabeled estradiol, [2,3,6,7,16,17-^{3}H(N)]-estradiol

TEDG: Assay buffer (10 mM Tris, 1.5 mM EDTA, 1 mM dithiothreitol, 10% glycerol, pH 7.4)

HAP: Hydroxylapatite

DES: Diethylstilbestrol

I. Preparation of Rat Uterine Cytosol

a. Prepare TEDG buffer (10 mM Tris, 1.5 mM EDTA, 1 mM dithiothreitol, 10% glycerol, pH 7.4). Dithiothreitol should be added just prior to use.

b. Uterine cytosol should be prepared using uteri from female rats ovariectomized 7 -10 days prior to being killed. Consistency should be maintained for all assays with respect to the age and strain of the females. Fat and mesentary should be quickly trimmed from the uterus. Weigh and record weight of each uterus. Uteri may be used immediately or rapidly frozen on dry ice or in liquid nitrogen, and stored at -80°C for up to 3 months.

c. Prepare uteri for homogenization using ice-cold TEDG buffer at a ratio of 0.1 g of tissue (e.g., use trimmed tissue necropsy weight obtained in step 1b) per 1.0 mL TEDG buffer.

d. Homogenize the tissue using a Polytron homogenizer (5-sec bursts). *Note:* Probe of polytron should be cooled prior to homogenizing each sample by placing the probe in ice-cold TEDG buffer. If possible, the homogenization tube should be kept in an ice-cold water bath during the homogenizing process.

e. Transfer the homogenate to pre-cooled centrifuge tubes and centrifuge for 10 min. at 2,500 x g at 4°C. (The pellet will contain the nuclear fraction and the supernatant will be used for the cytosolic preparation).

f. Transfer the supernatant to pre-cooled ultracentrifuge tubes, balance the tubes and centrifuge at 105,000 x g for 60 min. at 4°C.

g. Combine the supernatant (i.e., cytosol containing ER) and aliquot for immediate use in ER binding assay or for freezing at -80°C. *Note:* cytosol can be frozen for 1 month prior to use in ER binding assay. Do not thaw and re-freeze the cytosol.

h. Determine the protein content for each batch of cytosol using the BioRad Protein Assay Kit (BioRad Chemical Division, Richmond, CA). *Note:* The dithiothreitol in the buffer is not compatible with the Pierce BCA Protein Assay. Typical protein values are 4 -7 mg/mL.

II. Standardization of Methods for ER Binding Assay

Prior to routinely conducting the ER competitive binding assays, the methods should be standardized within each laboratory. This may be accomplished in two steps. First, a series of saturation radioligand binding assays should be conducted to demonstrate ER specificity and saturation. Nonlinear regression analysis of these data and subsequent Scatchard plots will document ER binding affinity (k_d) and number (B_{max}). Second, a series of ER competitive binding assays should be conducted using chemicals with known affinities for the ER, such as inert E2, DES, estrone. Comparison of IC_{50}s (e.g., the concentration of a chemical that inhibits ^{3}H-E2 binding by 50%)from these assays with reported values in the literature will assist in documenting that the methods are appropriate for routine use in the laboratory.

A. Saturation Radioligand Binding Assay. ER saturation binding experiments measure total, non-specific and specific binding of increasing concentrations of ^{3}H-E2 under conditions of equilibrium. A graph of specific ^{3}H-E2 binding vs. radioligand concentration should reach a plateau for maximum specific binding indicative of saturation of the ER with the radioligand. In addition, analysis of the data should document the binding of the ^{3}H-E2 to a single, high affinity binding site (e.g, K_d = 0.05 - 0.1 nM).

Although several saturation radioligand assays may need to be conducted before an optimal saturation curve, k_d and B_{max} are achieved, a good starting point is to use enough cytosol to provide 50 - 100 µg protein per assay tube. The concentration for ^{3}H-E2 should range from 0.03 - 3.0 nM in a total assay volume of 0.5 mL. Non-specific binding should be determined by using 100 x the concentration of radiolabeled E2. Analysis of these data should use a non-linear regression analysis such as RADLIG and LIGAND (KELL, BioSoft, Ferguson, MO), with a final display of the data as a Scatchard plot. Rat uterine cytosol prepared using this protocol will typically yield a k_d of 0.05 - 0.1 nM and B_{max} of 36 -44 fmol ER/100 ug protein (e.g, 0.072 - 0.088 nM ER when 100 µg protein used in total assay volume of 0.5 mL).

An example of a saturation assay worksheet using increasing concentrations of radioligand is shown below. *Note:* For this example, a stock solution of inert E2 should be prepared in ethanol, with all serial dilutions prepared in assay buffer. All concentrations of ^{3}H-E2 should be prepared in assay buffer.

Typical Estradiol Saturation Assay									
	^{3}H-E2			***Inert E2***			***Buffer***	***Cytosol***	
Tube #	***Initial [ ] nM***	***Vol (µl)***	***Final [ ] nM***	***Initial [] nM***	***Vol (µl)***	***Final [ ] nM***	***Vol (µl)***	***Vol (µl)***	***DPM***
1	0.3	50	0.03	-			350	100	
2	0.3	50	0.03	-			350	100	
3	0.6	50	0.06	-			350	100	
4	0.6	50	0.06	-			350	100	
5	0.8	50	0.08	-			350	100	
6	0.8	50	0.08	-			350	100	
7	1.0	50	0.1	-			350	100	
8	1.0	50	0.1	-			350	100	
9	3.0	50	0.3	-			350	100	
10	3.0	50	0.3	-			350	100	
11	6.0	50	0.6	-			350	100	
12	6.0	50	0.6	-			350	100	
13	10	50	1	-			350	100	
14	10	50	1	-			350	100	
15	30	50	3	-			350	100	
16	30	50	3	-			350	100	
17	0.3	50	0.03	30	50	3	300	100	
18	0.3	50	0.03	30	50	3	300	100	
19	0.6	50	0.06	60	50	6	300	100	
20	0.6	50	0.06	60	50	6	300	100	
21	0.8	50	0.08	80	50	8	300	100	
22	0.8	50	0.08	80	50	8	300	100	
23	1.0	50	0.1	100	50	10	300	100	
24	1.0	50	0.1	100	50	10	300	100	
25	3.0	50	0.3	300	50	30	300	100	
26	3.0	50	0.3	300	50	30	300	100	
27	6.0	50	0.6	600	50	60	300	100	
28	6.0	50	0.6	600	50	60	300	100	
29	10	50	1	1000	50	100	300	100	
30	10	50	1	1000	50	100	300	100	
31	30	50	3	3000	50	300	300	100	
32	30	50	3	3000	50	300	300	100	
33	0.3	50	0.03	^{3}H- E2 only, for determining total dpms					
34	0.3	50	0.03	^{3}H- E2 only, for determining total dpms					
35	0.6	50	0.06	^{3}H- E2 only, for determining total dpms					
36	0.6	50	0.06	^{3}H- E2 only, for determining total dpms					
37	0.8	50	0.08	^{3}H- E2 only, for determining total dpms					

38	0.8	50	0.08	^{3}H- E2 only, for determining total dpms	
39	1.0	50	0.1	^{3}H- E2 only, for determining total dpms	
40	1.0	50	0.1	^{3}H- E2 only, for determining total dpms	
41	3.0	50	0.3	^{3}H- E2 only, for determining total dpms	
42	3.0	50	0.3	^{3}H- E2 only, for determining total dpms	
43	6.0	50	0.6	^{3}H- E2 only, for determining total dpms	
44	6.0	50	0.6	^{3}H- E2 only, for determining total dpms	
45	10	50	1	^{3}H- E2 only, for determining total dpms	
46	10	50	1	^{3}H- E2 only, for determining total dpms	
47	30	50	3	^{3}H- E2 only, for determining total dpms	
48	30	50	3	^{3}H- E2 only, for determining total dpms	

B. ER Competitive Binding Assay. An ER competitive binding assay measures the binding of a single concentration of ^{3}H-E2 in the presence of increasing concentrations of a test chemical. The competitive binding curve is plotted as total ^{3}H-E2 binding vs. the concentration (log units) of the competitor. The concentration of the test chemical that inhibits 50% of the maximum ^{3}H-E2 binding is the IC_{50}. Preliminary experiments should evaluate the effect of the ER concentration of the cytosol, assay volume and ^{3}H-E2 concentration on the IC_{50} calculation using inert E2. A good starting point for the ER competitive binding assay is to use enough cytosol to provide 50 - 100 µg protein per assay tube, with 0.5 -1.0 nM ^{3}H-E2 in a total assay volume of 0.5 mL. Suggested concentrations for test chemicals with a high affinity for the ER are 1×10^{-11} to 1×10^{-7} M; and 1×10^{-10} to 3×10^{-4} M for chemicals expected to have a lower binding affinity for the ER. Once assay conditions have been optimized, additional ER competitive binding assays should be conducted to compare chemicals with known affinities for the ER, such as DES, estrone, and ethynyl estradiol (positive controls), and the androgen agonist, R1881 (negative control). (See Pages 13 -14 of this protocol for Example Worksheet: ER Competitive Binding Assay). Data for the inert E2 standard curve and each test chemical should be plotted as the percent ^{3}H-E2 bound versus the molar concentration (log) of competitor. Estimates of IC_{50} should be determined using appropriate nonlinear curve fitting software such as GraphPad Prism (GraphPad Software, Inc., San Diego, CA). (See Pages 10 -12 of this protocol for additional comments on data analysis).

C. Checklist for Standardizing ER Binding Assays within Laboratory

i. Saturation Assays. If conducting the ER assay is new to the laboratory, several publications cited in the reference section of this protocol will be extremely useful when evaluating the data (e.g., Book edited by Hulme et. al., and the manuals from GraphPad Prism and Biosoft KELL). In general, when evaluating data from the ER saturation assays, the following points should be considered.

- As increasing concentrations of ^{3}H-E2 were used, did the specific binding curve reach a plateau? (e.g, Was maximum specific binding reached indicating saturation of ER with ligand?).
- Did the data produce a linear Scatchard plot? (e.g., Nonlinear plots generally indicate a problem with the assay such as ligand depletion (concave plot) or incorrect assessment of non-specific binding (convex plot), etc.).

- Is the K_d within an acceptable range (e.g., 0.05 - 0.1 nM)? *Note:* Literature values for K_d using uterine cytosolic preparations have varied from 0.05 - 0.5 nM. The variation in K_d may be a reflection of different labs using radiolabeled estradiol with a wide range of specific activity (^{3}H-E2 vs ^{125}I-E2). In addition, publications by Salomonsson et al. (1994) and Kuiper et al. (1997, 1998) suggest that a lower Kd may be observed when assays conditions minimize ligand depletion, and that slightly different K_ds exist for ERα and ERβ.
- Are the standard errors for the K_d or B_{max} too high? Divide the standard error (SE) of the k_d by the k_d, and the SE of the B_{max} by the B_{max}. If either ratio is much larger than 20%, then the methods for the assay should be re-evaluated (GraphPad Prism Manual, 1999).
- Is non-specific binding too high? The value for non-specific binding should be less than 50% of the total binding (GraphPad Prism Manual, 1999).

ii. Competitive Binding Assays. Again, if the assay is new to the laboratory, it is suggested that the publications cited in the reference section be utilized to facilitate adequate evaluation of the data. In general, the assay should demonstrate that increasing concentrations of inert E2 can compete with a single concentration of ^{3}H-ER for binding to the ER. Specific questions to evaluate are as follows:

- As a safeguard against ligand depletion, was the total maximal binding no greater than 10% of the amount of ^{3}H-E2 added per assay tube?
- Was the IC_{50} for inert E2 reasonable? (e.g The IC_{50} for inert E2 should be approximately equal to the molar concentration of ^{3}H-E2 used in the assay tube plus the K_d (determined by nonlinear analysis and Scatchard plot of data obtained from saturation radioligand binding assays).
- Are the data easily replicated with respect to IC_{50}s for inert E2, and selected test chemicals?

III. Estrogen Receptor Competitive Binding Assay: Working Protocol

1.0 Preparation of Assay Buffer

TEDG Buffer (10 mM Tris, 1.5 mM EDTA, 1.0 mM dithiothreitol, 10% glycerol).
Prepare buffer without dithiothreitol, adjust to pH 7.4 and store at 4^o C.
Add dithiothreitol just prior to use in assay.

2.0 Preparation of Trace

[2,3,6,7,16,17-^{3}H(N)]-estradiol (^{3}H-E2)
New England Nuclear (DuPont) No. NET-517
Store at 4 - 5 ^{o} C in original container.
Specific Activity (SA) may change with lot.
Obtain the highest specific activity available from the vendor.
Example: Lot # 33639215, Certification Date 5/16/01;
SA = 118 Ci/mmol (261,960 DPM/pmol).
Concentration: 1.0 mCi/mL
Note: SA should be adjusted for decay over time.

Dilute trace with TEDG assay buffer.
Each assay tube should contain 0.5 - 1 nM final concentration of ^{3}H-E2.

3.0 Selection of Receptor Concentration and Assay Volume

a. Receptor concentration of the cytosol and assay volume per assay tube should be adjusted to minimize the likelihood of ligand depletion (e.g., Ligand depletion occurs when a high percentage of the ^{3}H-E2 is bound to ER causing the concentration of the unbound (*free*) ^{3}H-E2 to significantly differ from the concentration of ^{3}H-E2 that was originally added to the assay tube. Hulme and Birdshall, 1992). A general rule of thumb is to optimize the assay conditions so that the ratio of the total ^{3}H-E2 bound in the absence of competitor, to the total ^{3}H-E2 added to each assay tube, is no more than 10%. Decreasing the amount of cytosolic protein and/or increasing the assay volume will generally lower this ratio. Serial dilutions of the cytosol to obtain 50 - 150 µg protein per assay tube in a total assay volume of 500 µL is a good starting point for determining the optimal ER concentration.

4.0 Preparation of E2 for the Standard Curve and nonspecific binding (NSB).

a. Standard Curve: A standard curve using inert E2 should be prepared for each ER competitive binding assay. Final concentrations of inert E2 in the assay tubes should range from 1.0×10^{-7} to 1.0×10^{-11} M. Prepare serial dilutions of E2 in ethanol (200 proof) to achieve the Final Concentrations shown below. Use siliconized glass tubes when preparing the standards. The following table shows recommended concentrations for the inert E2 standard curve.

Example of Preparation Procedure for Inert E2 Standard Curve

Concentrations for Inert E2 Standard Curve		
Standards	***Initial E2 Concentration (Molar)***	****Final E2 Concentration (Molar) in ER assay tube***
Negative Control	0 (Inert R1881)	0
0	0 (EtOH)	0
NSB	5×10^{-6}	1×10^{-7}
S1	5×10^{-7}	1×10^{-8}
S2	5×10^{-8}	1×10^{-9}
S3	1.67×10^{-8}	3.33×10^{-10}
S4	5×10^{-9}	1×10^{-10}
S5	1.67×10^{-9}	3.33×10^{-11}
S6	5×10^{-10}	1×10^{-11}
** When 10 µl of each standard is added to the ER assay tube, the final concentration will be as indicated when the total volume in the ER assay tube is 500 µl.*		

b. Negative control: It is recommended a chemical be selected as a negative control (e.g., does not demonstrate any binding affinity for the ER), and one concentration of the chemical be

tested in each competitive binding assay. R1881, an androgen agonist, at a final concentration of 1 x 10^{-7} M is used in this protocol as the negative control.

5.0 Preparation of Test Chemicals

a. Stock Solutions: Test chemicals should be diluted in ethanol (200 proof) to 3.0 x 10^{-2} M (i.e., 30 mM). Use siliconized glass tubes when preparing dilutions. *Note:* Some test chemicals will not be soluble at this concentration, so adjustments will need to be made in the final concentration of the serial dilution tubes depending upon the specific chemical. Likewise, some chemicals may not be soluble in ethanol at all, so appropriate modifications in the ER assay should be made to accommodate any change in solvent.

b. Prepare serial dilutions of each test chemical in ethanol to yield the final concentrations as indicated below. *Note:* The serial dilutions shown in Table 2 are based upon the addition of 10 µl of each serial dilution of the test chemical in a final assay volume of 500 µl. *Caution:* No more than 0.2% ethanol should be used in the assay tubes.

Table 2 – Test Chemical Concentrations

Serial Dilutions of Test Chemical	***Initial Concentration (Molar)***	****Final Concentration in ER assay tube (Molar)***
Concentration 1	15 x 10^{-3}	3.0 x 10^{-4}
Concentration 2	5.0 x 10^{-3}	1.0 x 10^{-4}
Concentration 3	5.0 x 10^{-4}	1.0 x 10^{-5}
Concentration 4	5.0 x 10^{-5}	1.0 x 10^{-6}
Concentration 5	5.0 x 10^{-6}	1.0 x 10^{-7}
Concentration 6	5.0 x 10^{-7}	1.0 x 10^{-8}
Concentration 7	5.0 x 10^{-8}	1.0 x 10^{-9}
Concentration 8	5.0 x 10^{-9}	1.0 x 10^{-10}

**Final Concentration of test chemical in assay tube when 10 µl of Initial Concentration is used in a total volume of 500 µl.*

6.0 Preparation of ER Assay Tubes

a. Label 12 x 75 mm round bottom assay tubes (siliconized glass) in duplicate as follows:

0, NSB, Neg, S1, S2, S3, S4, S5, S6
Unknown chemical 1- Concentration 1 (e.g., U1-C1, U1-C2, ... U1-C8)
Unknown chemical 2 -Concentration 1 (e.g., U2-C1, U2-C2, ...U2-C8)
Total DPMS: TC

b. Place assay tubes in ice bath and add the following to each tube:

Components of ER Competitive Binding Assay		
50	µL	Adjust amount of uterine cytosol to provide 50 - 100 µg protein/assay tube
430	µL	TEDG Assay Buffer
10	µL	^{3}H-E2 to yield final concentration of 0.5 - 1.0 nM
10	µL	Inert E2, negative control, or test chemical
500	µL	Total volume in each assay tube

c. Vortex assay tubes. (*Note:* Make sure that all components are concentrated at the bottom of tube. If any of the liquid remains on the side of the tube, centrifuge assay tubes for 1 minute at 600 x g (4^{o}C) to concentrate fluid at bottom of tube.)

d. Incubate assay tubes at 4^{o} C for 18 to 20 h. Assay tubes should be placed on a rotator during the incubation period.

6.0 Preparation of 60% Hydroxylapatite (HAP) Slurry

a. The day before beginning this step to separate the bound and free ^{3}H-E2, add 10 g HAP (BioRad) to 100 mL TEDG buffer and gently mix. Cap the container and place the HAP slurry in the refrigerator overnight. (This amount of HAP will generally yield enough slurry for 70 - 100 assay tubes.)

b. The next morning aspirate the supernatant and resuspend the HAP in fresh TEDG buffer. Allow HAP to settle and repeat wash. If HAP is prepared in a graduated cylinder, the amount of buffer needed to prepare a 60% HAP slurry can be estimated using the scale on the outside of the cylinder.

c. After the last wash, resuspend the HAP to a final volume of 60% HAP and 40% buffer. The HAP slurry should be well suspended and ice cold when used in the separation procedure.

7.0 Separation of Bound ^{3}H-E2-ER and Free ^{3}H-E2

Note: To minimize dissociation of bound ^{3}H-E2 from the ER during this process, it is extremely important that the buffers and assay tubes be kept ice-cold and that each step be conducted quickly.

a. Remove ER assay tubes from rotator and place in an ice-water bath. Using an Eppendorf repeating pipet, quickly add 250 µL of HAP slurry (60% in TEDG buffer, well mixed prior to using) to each assay tube.

b. Vortex the tubes at 5 minute intervals for a total of 15 minutes. (*Note:* This is best accomplished by vortexing an entire rack of tubes at once. It is important to keep the assay tubes cold at this point.)

c. Following the incubation period (step 7b), add 2.0 mL of the TEDG assay buffer, quickly vortex, and centrifuge at 4^{o}C for 10 minutes at 1000 x g.

d. At the end of the centrifugation, immediately decant the supernatant (e.g., containing the free ^{3}H-E2. The HAP pellet will contain the bound ^{3}H-E2-estrogen receptors). *Note:* This step can be accomplished quickly by placing the assay tubes in a decanting tube racks. All tubes in the rack can be decanted at once, and the tubes immediately placed back in the ice bath.

e. Add an additional 2.0 mL TEDG ice-cold buffer and vortex briefly to resuspend pellet. Work quickly and keep assay tubes cold. Centrifuge again at 4^{o}C for 15 minutes at 1000 x g.

f. Again quickly decant and discard supernatant. Repeat the wash and centrifugation steps once more.

g. After the final wash, decant the supernatant. Allow the assay tubes to drain briefly for 1 - 5 minutes. (*Note:* Watch carefully in case the HAP pellet begins to run down the side of assay tube which may occur if protein concentration in the cytosol is quite low.) At this point, the separation of the free 3H-E2 and bound ^{3}H-E2-ER has been completed. Assay tubes may be left at room temperature.

8.0 Extraction and Quantifying ^{3}H-E2 bound to ER

a. Add 1.5 ml of ethanol (100%) to each assay tube. Allow the tubes to sit at room temperature for 15 - 20 minutes, vortexing at 5 minute intervals.

b. Centrifuge the assay tubes for 10 minutes at 1000 x g. (Centrifuge can be set at 4^{o}C, but keeping the assay tubes cold is no longer critical at this point.)

c. Pipet an aliquot (usually 1.0 -1.5 ml) or decant the supernatant into 20 mL scintillation vials. Add 10 mL scintillation cocktail, cap and shake vial. *Note:* If a 1.0 ml aliquot is used for scintillation counting, the DPMs should be adjusted to account for the total radioactivity in 1.5 ml (e.g., DPMs x 1.5 = Total DPMs bound).

d. Place vials in scintillation counter for determination of DPMs/vial with quench correction.

9.0 Data Analysis

a. Terminology:

Total ^{3}H-E2: DPMs added to each assay tube
(e.g., can be converted to concentration of total ^{3}H-E2 used in the ER assay)
Total (Maximum) Binding: DPMs in the 0 standard tubes.
Nonspecific Binding: DPMs in the NSB standard (i.e., 100 x excess of inert E2)
Specific Binding: DPMs for each concentration of standard or test chemical minus the mean DPM of the NSB tubes.

b. Data Analysis:

i. IC_{50} calculation: Data for the inert E2 standard curve and each test chemical should be plotted as the percent ^{3}H-E2 bound versus the molar concentration (log) of competitor. Estimates of IC_{50} should be determined using appropriate nonlinear curve fitting software such as GraphPad Prism (GraphPad Software, Inc., San Diego, CA).

ii. Relative Binding Affinity (RBA): The RBA for each competitor (test chemical) should be calculated by dividing the IC_{50} for E2 by the IC_{50} of the competitor and expressing as a percent (e.g., RBA for E2 =100 %).

c. Checklist for Evaluating ER Competitive Binding Assay Data

i. Inert E2 Standard Curve. The assay should demonstrate that increasing concentrations of inert E2 can displace ^{3}H-E2. The IC_{50} for E2 should be approximately equal to the molar concentration of ^{3}H-E2 plus the K_d (determined by Scatchard analysis). (Prism, GraphPad). The IC_{50}s for the inert E2 standard curve should be easily replicated.

ii. IC_{50}s for test chemicals should be easily replicated.

iii. The ratio of total binding in the absence of competitor to the amount of ^{3}H-E2 added per assay tube should not be greater than 10%.

IV. References

Korach KS and Muldoon TG, (1974). Studies on the nature of the hypothalamic estradiol-concentrating mechanism in the male and female rat. Endocrinology 94: 785 - 793

Laws SC, Carey SA, Hart DW, Cooper RL (1994). Lindane does not alter the estrogen receptor or the estrogen-dependent induction of progesterone receptors in sexually immature or ovariectomized adult rats. Toxicology 92:127-142.

Blair RM, Fang H, Branham WS, Hass BS, Dial SL, Moland CL, Tong W., Shi L, Perkins R. Sheehan DM. (2000). The estrogen receptor relative binding affinities of 100 natural and xenochemicals: structural diversity of ligands. Tox. Sciences 54:138-153.

Laws SC, Carey SA, Ferrell JM, Bodman GJ, Cooper RL. (2000). Estrogenic activity of octylphenol, nonylphenol, bisphenol A and methoxychlor in rats. Tox. Sciences 54:154-167.

Kuiper G, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson J. (1997). Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology 138(3):863-870.

Kuiper G, Lemmen J, Carlsson B, Corton JC, Safe S, Van Der Saag P, Van Der Burg B, Gustafsson J. (1998). Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. Endocrinology 139(10): 4252-4263.

Salomonsson M, Carlsson B, Haggblad J. (1994). Equilibrium hormone binding to human estrogen receptors in highly diluted cell extracts is non-cooperative and has a Kd of approximately 10 pM. J. Steroid Biochem. Molec. Biol. 50(5/6):313-318.

Hulme and Birdshall. (1992). Strategy and tactics in receptor-binding studies. In: Receptor Ligand Interactions: A Practical Approach. Ed. E.C. Hulme. IRL Press, New York. pp. 63-176.

Motulsky HJ. (1999). Analyzing Data with GraphPad Prism, GraphPad Software Inc., San Diego CA, http://www.graphpad.com.

McPherson GA. (1985). Analysis of radioligand binding experiments. A collection of computer programs for the IBM PC. J Pharmacol Methods. Nov 14(3):213-228.

McPherson, GA. (c1997). BioSoft KELL: A collection of programs for the analysis of radioligand binding experiments (KINETIC, EBDA, LIGAND, LOWRY for Windows), Ferguson, MO; http://www.biosoft.com.

Example worksheet: Includes a standard curve, a test chemical, and quality control measures

Date and time of assay:_____ Person conducting the assay:_____

Radioligand: ^{3}H-E2 lot number:_____, specific activity: _____Ci/mmole, _____ DPM/pmol,

Uterine Cytosol batch number _____, Protein concentration _____mg/mL ,
Amt. Protein used per assay vial: _____ug,

Date uteri harvested_____ : Date of cytosol preparation_____

Posn and tube label	*Competitor Concentration*	*Competitor*	*Initial Concentration (Molar)*	*Receptor (ul)*	*Buffer*	*Tracer*	*Competitor*	*HAP*	*Final (Molar)*
1	0	EtOH		50	430	10	10	250	
2	0	EtOH		50	430	10	10	250	
3	NSB	Inert E2	5E-6	50	430	10	10	250	1E-7
4	NSB	Inert E2	5E-6	50	430	10	10	250	1E-7
5	S1	Inert E2	5E-7	50	430	10	10	250	1E-8
6	S1	Inert E2	5E-7	50	430	10	10	250	1E-8
7	S2	Inert E2	5E-8	50	430	10	10	250	1E-9
8	S2	Inert E2	5E-8	50	430	10	10	250	1E-9
9	S3	Inert E2	1.67E-8	50	430	10	10	250	3.33E-10
10	S3	Inert E2	1.67E-8	50	430	10	10	250	3.33E-10
11	S4	Inert E2	5E-9	50	430	10	10	250	1E-10
12	S4	Inert E2	5E-9	50	430	10	10	250	1E-10
13	S5	Inert E2	1.67E-9	50	430	10	10	250	3.33E-11
14	S5	Inert E2	1.67E-9	50	430	10	10	250	3.33E-11
15	S6.	Inert E2	5E-10	50	430	10	10	250	1E-11
16	S6	Inert E2	5E-10	50	430	10	10	250	1E-11
17	Neg.	Inert R1881	5E-6	50	430	10	10	250	1E-7
18	Neg.	Inert R1881	5E-6	50	430	10	10	250	1E-7
19	u1-c1	Chemical 1	15E-3	50	430	10	10	250	3E-4
20	u1-c1	Chemical 1	15E-3	50	430	10	10	250	3E-4
21	u1-c2	Chemical 1	5E-3	50	430	10	10	250	1E-4
22	u1-c2	Chemical 1	5E-3	50	430	10	10	250	1E-4
23	u1-c3	Chemical 1	5E-4	50	430	10	10	250	1E-5
24	u1-c3	Chemical 1	5E-4	50	430	10	10	250	1E-5
25	u1-c4	Chemical 1	5E-5	50	430	10	10	250	1E-6
26	u1-c4	Chemical 1	5E-5	50	430	10	10	250	1E-6
27	u1-c5	Chemical 1	5E-6	50	430	10	10	250	1E-7
28	u1-c5	Chemical 1	5E-6	50	430	10	10	250	1E-7
29	u1-c6	Chemical 1	5E-7	50	430	10	10	250	1E-8
30	u1-c6	Chemical 1	5E-7	50	430	10	10	250	1E-8
31	u1-c7	Chemical 1	5E-8	50	430	10	10	250	1E-9
32	u1-c7	Chemical 1	5E-8	50	430	10	10	250	1E-9
33	u1-c8	Chemical 1	5E-9	50	430	10	10	250	1E-10
39	u1-c8	Chemical 1	5E-9	50	430	10	10	250	1E-10
40	0	EtOH		50	430	10	10	250	
41	0	EtOH		50	430	10	10	250	
42	NSB	Inert E2	5E-6	50	430	10	10	250	1E-7
43	NSB	Inert E2	5E-6	50	430	10	10	250	1E-7
44	Neg.	Inert R1881	5E-6	50	430	10	10	250	1E-7
45	Neg.	Inert R1881	5E-6	50	430	10	10	250	1E-7
46	Hot	Total Counts		—	—	10	—	—	
47	Hot	Total Counts		—	—	10	—	—	
48	Hot	Total Counts		—	—	10	—	—	
49	Hot	Total Counts		—	—	10	—	—	
50	Hot	Total Counts		—	—	10	—	—	

Appendix C

Chemical and Product Class Information for the Substances Tested in the *In Vitro* ER Binding Assays

Information Sorted by Substance Name

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Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
4,4'-(1,3-Adamantanediyl)diphenol			Phenol	
2-(1-Adamantyl)-4-methylphenol		41031-50-9	Phenol	
4-(1-Adamantyl)phenol		29799-07-3	Phenol	
Alachlor		15972-60-8	Anilide	Pesticide
Aldosterone		52-39-1	Steroid, nonphenolic	Pharmaceutical
Aldrin		309-00-2	Cyclodiene	Pesticide
δ-*trans* Allethrin		584-79-2	Pyrethrin; Pyrethroid	Pesticide
p -(7-Alloxyl)-11-ethyldibenzo-[*b,f*]thiepin-10-yl)phenol		85850-86-8	Stilbene; Phenol	
p -(3-(Alloxyl)-11-ethyl-6*H* -dibenzo[*b,f*]thiocin-12-yl)phenol hemihydrate		85850-88-0	Triphenylethylene; Phenol	
p -(2-(Alloxyl)-6-ethyl-11,12-dihydroxydibenzo[*a,e*]cyclooctene-5-yl)phenol		85850-87-9	Triphenylethylene; Phenol	
3-(Alloxyl)-10-ethyl-11-(4-hydroxyphenyl)dibenz[*b,f*]thiepin		85850-85-7	Triphenylethylene	
3-(Alloxyl)-10-ethyl-11-phenyldibenzo[*b,f*]thiepin		85850-82-4	Triphenylethylene	
3-(Alloxyl)-11-ethyl-12-phenyl 6*H* -dibenzo[*b,f*]thiocin		85850-84-6	Triphenylethylene	
3-(Alloxyl)-10-ethyl-11-phenyldibenz[*b,f*]oxepin		83807-07-2	Triphenylethylene	
3-(Alloxyl)-11-ethyl-12-phenyl 5,6-dihydroxydibenz[*a,e*]cyclooctene		85850-83-5	Triphenylethylene	
Amaranth	Acid red 27	915-67-3	Azo compound	Dye
2-Aminoestratriene-3,17β-diol	2-Aminoestradiol	107900-30-1	Steroid, phenolic	
4-Aminoestratriene-3,17β-diol	4-Aminoestradiol	107900-31-2	Steroid, phenolic	
2-Aminoestratrien-17β-ol		17522-06-4	Steroid, nonphenolic	
4-Aminoestratrien-17β-ol		17522-04-2	Steroid, nonphenolic	
4-Aminophenyl ether	4,4'-Oxydianiline	101-80-4	Aniline	
4-*tert* -Amylphenol	4-*tert* -Pentylphenol	80-46-6	Alkylphenol	Chemical intermediate
3β-Androstanediol		25126-76-5	Steroid, nonphenolic	
5α-Androstane-3α,17β-diol	3α-Androstanediol	1852-53-5	Steroid, nonphenolic	Pharmaceutical
5α-Androstane-3β,17β-diol	3β-Androstanediol	571-20-0	Steroid, nonphenolic	Pharmaceutical
5β-Androstane-3α,17β-diol		1851-23-6	Steroid, nonphenolic	Pharmaceutical
5β-Androstanedione	5β-Androstane-3,17-dione	1229-12-5	Steroid, nonphenolic	Pharmaceutical
5β-Androstane-3,17-dione	5α-Androstanedione	5982-99-0	Steroid, nonphenolic	
5α-Androstane-3α-ol-17-one		53-41-8	Steroid, nonphenolic	Pharmaceutical
4-Androstenediol	3β,17β-Dihydroxy-4-androstene; Androst-4-ene-3β,17β-diol	1156-92-9	Steroid, nonphenolic	Pharmaceutical

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
5-Androstenediol	3β,17β-Dihydroxy-5-androstene; Androst-5-ene-3β,17β-diol; 5-Androstene-3β,17β-diol	521-17-5	Steroid, nonphenolic	Pharmaceutical
4-Androstenedione	4-Androstene-3,17-dione	63-05-8	Steroid, nonphenolic	Precursor of testosterone and other hormones
Anthracene		120-12-7	Polycyclic aromatic hydrocarbon	None found (air pollutant)
Apigenin		520-36-5	Flavone	Natural product
Aroclor 1221		11104-28-2	Polychlorinated biphenyl	Dielectric fluid
Aroclor 1254		11097-69-1	Polychlorinated biphenyl	Dielectric fluid
Atrazine		1912-24-9	Triazine; Aromatic amine	Pesticide
Aurin	Aurine; Corallin	603-45-2	Diphenolalkane	Chemical intermediate
Baicalein		491-67-8	Flavone	Natural product
Benomyl		17804-35-2	Carbamate; Imidazole	Pesticide
Benz[*a*]anthracene	1,2-Benzanthracene	56-55-3	Polycyclic aromatic hydrocarbon	
Benzeneacetonitrile α-[bis(4-hydroxyphenyl) methylene]		66422-14-8	Diphenolalkane; Bisphenol	Chemical intermediate
Benzo[*a*]carbazole		239-01-0	Polycyclic aromatic hydrocarbon	
Benzo[*c*]carbazole			Polycyclic aromatic hydrocarbon	
Benzo[*b*]fluoranthene		205-99-2	Polycyclic aromatic hydrocarbon	
Benzo[*k*]fluoranthene		207-08-9	Polycyclic aromatic hydrocarbon	
Benzo[*a*]fluorene	1,2-Benzofluorene; Chrysofluorene	238-84-6	Polycyclic aromatic hydrocarbon	
Benzo[*b*]fluorene		243-17-4	Polycyclic aromatic hydrocarbon	
Benzo[*b*]naptho[2,1-d]thiophene		239-35-0	Polycyclic aromatic hydrocarbon	
Benzo[*b*]naptho[2,3-d]thiophene		243-46-9	Polycyclic aromatic hydrocarbon	
Benzo[*ghi*]perylene	1,12-Benzoperylene	191-24-2	Polycyclic aromatic hydrocarbon	
Benzo[*c*]phenanthrene		195-19-7	Polycyclic aromatic hydrocarbon	
Benzo[*a*]pyrene	3,4-Benzopyrene	50-32-8	Polycyclic aromatic hydrocarbon	
Benzo[*e*]pyrene	1,2-Benzopyrene; 4,5-Benzopyrene	192-97-2	Polycyclic aromatic hydrocarbon	
Benzyl alcohol	Benzenemethanol	100-51-6	Alcohol	
4-Benzyloxyphenol	Benzyl 4-hydroxyphenyl ether; Benzoquin	103-16-2	Phenol	Pharmaceutical

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
Benzylparaben	Benzyl 4-hydroxybenzoate; Benzyl *p* -hydroxybenzoate	94-18-8	Paraben	
Biochanin A		491-80-5	Isoflavone	
Bis(*m* -acetoxy)-1,1,2-triphenylbut-1-ene		100808-56-8	Stilbene	
Bis(*p* -acetoxy)-1,1,2-triphenylbut-1-ene		100808-54-6	Triphenylethylene	
Bisdesoxyestradiol	Estratriene	1217-09-0	Steroid, nonphenolic	
1,1-Bis-(4-hydroxyphenyl) ethane		2081-08-5	Diphenolalkane	Chemical intermediate
4,4-Bis(4-hydroxyphenyl)heptane		7425-79-8	Diphenolalkane	Chemical intermediate
3,4-Bis(3-hydroxyphenyl)hexane		68266-24-0	Diphenolalkane	Chemical intermediate
3,3-Bis(4-hydroxyphenyl)pentane		3600-64-4	Diphenolalkane; Bisphenol	Chemical intermediate
1,1-Bis(4-hydroxyphenyl)propane		1576-13-2	Diphenolalkane; Bisphenol	Chemical intermediate
2,2-Bis(4-hydroxyphenyl)propanol		142648-65-5	Bisphenol	Chemical intermediate
2,2-Bis(*p* -hydroxyphenyl)-1,1,1-trichloroethane	2,2-Bis(4-hydroxyphenyl)-1,1,1-trichloroethane; Dihydroxymethoxychlor	2971-36-0	Organochlorine; Bisphenol	Pesticide metabolite
Bisphenol A	4,4'-Isopropylidenediphenol; 4,4'-(1-Methylethylidene)bisphenol	80-05-7	Bisphenol	Chemical intermediate
Bisphenol A bis(chloroformate)	BPACF	2024-88-6	Bisphenol	Chemical intermediate
Bisphenol A diglycidyl ether	BADGE	1675-54-3	Bisphenol	Chemical intermediate
Bisphenol A diglycidyl ether dimethacrylate	BisGMA	1565-94-2	Acrylate	Chemical intermediate
Bisphenol A dimethacrylate		3253-39-2	Acrylate; Bisphenol	Chemical intermediate
Bisphenol A ethoxylate	E-BPA	68140-85-2	Polyether	Chemical intermediate
Bisphenol A ethoxylate diacrylate	BPA-EDA	64401-02-1	Acrylate	Chemical intermediate
Bisphenol A glucuronide			Bisphenol; Glucuronide	
Bisphenol A propoxylate	P-BPA	37353-75-6	Bisphenol	
Bisphenol AF	2,2-Bis(4-hydroxyphenyl)perfluoropropane	1478-61-1	Diphenolalkane; Bisphenol	Chemical intermediate
Bisphenol B	2,2-Bis(4-hydroxyphenyl)butane	77-40-7	Diphenolalkane; Bisphenol	Chemical intermediate
Bisphenol C	2,2-Bis(4-hydroxy-3-methylphenyl)propane	79-97-0	Diphenolalkane; Bisphenol	Chemical intermediate
Bisphenol C 2		14868-03-2	Diphenolalkane; Bisphenol	Chemical intermediate
Bisphenol E	1,1-Bis(4-hydroxyphenyl)ethane; 4,4'-Ethylenediphenol	6052-84-2	Diphenolalkane; Bisphenol	Chemical intermediate
2,2'-Bisphenol F	Bis(2-hydroxyphenyl)methane	2467-02-9	Bisphenol	Chemical intermediate
4,4'-Bisphenol F		620-92-8	Phenol; Bisphenol	Chemical intermediate
Bisphenol S	4,4'-Sulfonyldiphenol	80-09-1	Bisphenol	Chemical intermediate
16α-Bromo-17β-estradiol		54982-79-5	Steroid, phenolic	

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
1,3-Butanediol, 4-[4-(1,2,3,4-tetrahydro-6-hydroxy-2-phenyl-1-naphthalenyl)phenoxy]-		107144-85-4	Phenyl ether	
1,3-Butanediol, 4-[4-(1,2,3,4-tetrahydro-6-methoxy-2-phenyl-1-naphthenyl)phenoxy]-		107163-56-4	Phenyl ether	
Butolame		150748-23-5	Steroid, phenolic	
Butyl 4-aminobenzoate		94-25-7	Aniline	Pharmaceutical
n -Butylbenzene		104-51-8	Aromatic hydrocarbon; Alkylbenzene	
sec -Butylbenzene	Benzene, (1-methylpropyl)-	135-98-8	Alkylbenzene	Chemical intermediate
Butyl benzyl phthalate	Benzyl butyl phthalate; *n* -Butyl benzyl phthalate; Butylbenzyl phthalate ester	85-68-7	Phthalate	Plasticizer
Butylparaben	Butyl 4-hydroxybenzoate; Butyl *p* -hydroxybenzoate	94-26-8	Paraben	Food additive; Pharmaceutical additive
2-*sec* -Butylphenol	*o-sec* -Butylphenol	89-72-5	Phenol	
2-*tert* -Butylphenol		88-18-6	Phenol	
3-*tert* -Butylphenol		585-34-2	Phenol	
4-*sec* -Butylphenol		99-71-8	Phenol	Pharmaceutical
4-*tert* -Butylphenol	*p-tert-* Butylphenol	98-54-4	Phenol	Chemical intermediate (coatings); Lubricant additive; Antioxidant (soap)
Butyl phthalyl *n* -butyl glycolate		85-70-1	Phthalate	Plasticizer
Caffeine		58-08-2	Purine	Pharmaceutical; Food additive; Natural product
Carbaryl	1-Naphthyl methylcarbamate	63-25-2	Carbamate; Polycyclic aromatic hydrocarbon	Pesticide
Carbofuran		1563-66-2	Carbamate	Pesticide
Castor oil		8001-79-4	Carboxylic acid	Pharmaceutical
(±)-Catechin		7295-85-4	Flavanone	
Chalcone		94-41-7	Chalconoid	Natural product
Chlordane		57-74-9	Organochlorine	Pesticide
α-Chlordane		5103-71-9	Organochlorine	Pesticide
Chlormequat chloride		999-81-5	Organochlorine	Plant growth regulator
2-Chloro-4-amino-6-isopropylamino-1,3,5 triazine		6190-65-4	Triazine	Pesticide
2'-Chloro-4,4'-biphenyldiol	2-Chloro-4,4'-biphenyldiol; 4,4'-dihydroxy-2'-chlorobiphenyl	56858-70-9	Organochlorine; Phenol	
2-Chloro-4-biphenylol	2-Chloro-4-hydroxybiphenyl; 4-hydroxy, 2-chloro biphenyl	23719-22-4	Organochlorine; Phenol	
4-Chloro-4'-biphenylol	4-Chloro-4'-hydroxybiphenyl	28034-99-3	Organochlorine; Phenol	
4-Chloro-*m* -cresol	4-Chloro-3-methylphenol	59-50-7	Organochlorine; Phenol	Preservative (Glue, gum, paint, ink, leather); Pesticide; Pharmaceutical

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
2-Chloro-4,6-diamino-*S* -triazine	Chlorodiaminotriazine 2 4 6 s	3397-62-4	Triazine	Pesticide
2-Chloro-4-ethylamino-6-amino-1,3,5-triazine		1007-28-9	Triazine	Pesticide
2-Chloro-4-ethylamino-6-(1-hydroxyisopropyl)amino-1,3,5-triazine		142179-80-4	Triazine	Pesticide
2-Chloro-4-isopropylamino-6-(1-hydroxyisopropylamino)-1,3,5-triazine		142200-36-0	Triazine	Pesticide
11β-Chloromethylestradiol		71794-60-0	Steroid, phenolic	
2-Chloro-4-methylphenol	2-Chloro-*p* -cresol; *p* -Cresol, 2-chloro-	6640-27-3	Chlorinated phenol	
4-Chloro-2-methylphenol	4-Chloro-*o* -cresol	1570-64-5	Organochlorine; Chlorinated phenol	Chemical intermediate
2-Chlorophenol	*o*- Chlorophenol	95-57-8	Chlorinated phenol	Chemical intermediate
4-Chlorophenol	*p* -Chlorophenol; *p*- Chlorophenic acid; 4-Hydroxychlorobenzene	106-48-9	Chlorinated phenol	Chemical intermediate
Chlorotamoxifen		77588-46-6	Triphenylethylene	
Cholesterol		57-88-5	Steroid, nonphenolic	Natural product (animal); Pharmaceutical
Chrysene	Benzo[*a*]phenanthrene; 1,2-Benzphenanthrene	218-01-9	Polycyclic aromatic hydrocarbon	
Chrysin		480-40-0	Flavone	Natural product
Cineole	1,8-Cineole; 1,8-Epoxy-*p* -menthane; *p* -Menthane, 1,8-epoxy Eucalyptol	470-82-6	Terpene	Fragrance
Cinnamic acid	2-Propenoic acid, 3-phenyl-	621-82-9	Carboxylic acid	Fragrance
cis -Clomiphene	Zuclomiphene	15690-55-8	Stilbene	Pharmaceutical
trans -Clomiphene	Clomiphene	911-45-5	Stilbene	Pharmaceutical
Clomiphene citrate		50-41-9	Triphenylethylene	Pharmaceutical
Colchicine		64-86-8	Amide	Pharmaceutical
Corticosterone	17-Deoxycortisol; 11,12-Dihydroxyprogesterone; 11β,21-Dihydroxyprogesterone; 11-Hydroxycorticoaldosterone	50-22-6	Steroid, nonphenolic	Pharmaceutical
Cortisol		50-23-7	Steroid, phenolic	Pharmaceutical
Coumestrol	2,(2,4-Dihydroxyphenyl)-6-hydroxy-3-benzofurancarboxylic acid δ-lactone	479-13-0	Coumarin; Phenol	Natural product (phytoestrogen)
p -Cumyl phenol		599-64-4	Phenol	Chemical intermediate
Cyclofenil diphenol		5189-40-2	Bisphenol	Pharmaceutical
Cycloprop[14*R* ,15α]estra-1,3,5(10)-triene-3,17β-diol, 3',15-dihydro-		73860-54-5	Steroid, phenolic	
Cycloprop[14*S* ,15β]estra-1,3,5(10)-triene-3,17β-diol, 3',15-dihydro-		105455-76-3	Steroid, phenolic	
Cypermethrin		52315-07-8	Organochlorine; Nitrile; Diphenyl ether	Pesticide
Daidzein	4',7-Dihydroxyisoflavone	486-66-8	Isoflavone	Natural product (phytoestrogen)

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
m,p '-DDD		4329-12-8	Organochlorine	Pesticide
o,p '-DDD	*o,p'* -TDE; Mitotane; 2,4'-Dichlorodiphenyldichloroethane	53-19-0	Organochlorine; Diphenylalkane	Pesticide; Pharmaceutical
p,p '-DDD	*p,p'* -TDE; 1,1-Dichloro-2,2-bis(*p* chlorophenyl)ethane	72-54-8	Organochlorine; Diphenylalkane	Pesticide
o,p '-DDE	1,1-Dichloro-2-(2-chlorophenyl)2-(4-chlorophenyl)ethylene	3424-82-6	Organochlorine; Diphenylalkane	Pesticide metabolite
p,p '-DDE	1,1-Dichloro-bis-(4-chlorophenyl)ethylene; 4,4'-DDE	72-55-9	Organochlorine; Diphenylalkane	Pesticide metabolite
o,p '-DDT		789-02-6	Organochlorine	Pesticide
(-)-*o,p* '-DDT		58633-26-4	Organochlorine	Pesticide
(+)-*o,p* '-DDT		58633-27-5	Organochlorine	Pesticide
p,p '-DDT	1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane	50-29-3	Organochlorine; Diphenylalkane	Pesticide
Dehydroepiandrosterone	Dehydroisoandrosterone; Androstenolone; 5-Androsten-3β-ol 17-one	53-43-0	Steroid, nonphenolic	Pharmaceutical
14-Dehydroestradiol-17β		58699-19-7	Steroid, phenolic	
9, 11-Dehydroestradiol			Steroid, phenolic	
14-Dehydroestradiol-17β 3-methyl ether		35664-58-7	Steroid, nonphenolic	
14-Dehydroestrone		2119-18-8	Steroid, phenolic	
14-Dehydroestrone 3-methyl ether		17550-11-7	Steroid, nonphenolic	
3-Deoxyestradiol	Estratriene-17β-ol	2529-64-8	Steroid, nonphenolic	
3-Deoxyestrone		53-45-2	Steroid, nonphenolic	Pharmaceutical
(*R*)-4'-Deoxyindenestrol A		138515-00-1	Stilbene	
(*rac*)-4'-Deoxyindenestrol A			Stilbene	
(*S*)-4'-Deoxyindenestrol A		138514-99-5	Stilbene	
(*R*)-5-Deoxyindenestrol A		138515-02-3	Stilbene	
(*rac*)-5-Deoxyindenestrol A		138472-84-1	Stilbene	
(*S*)-5-Deoxyindenestrol A		138515-01-2	Stilbene	
17-Desoxyestradiol	Estra-1,3,5(10)-triene-3-ol; Estratrien-3-ol; 17-Deoxyestrone	53-63-4	Steroid, phenolic	
Dexamethasone		50-02-2	Steroid, nonphenolic	Pharmaceutical
1,3-Diacetoxy-17α-ethinyl-7α-methyl-1,3,5(10)-estratrien-17β-ol			Steroid, nonphenolic	
4,4'-Diaminostilbene dihydrochloride		66635-40-3	Stilbene	
Dibenz[*ah*]anthracene		53-70-3	Polycyclic aromatic hydrocarbon	
Dibenzo-18-crown-6	Crown 18; Dibenzocrown; Dibenzo-18-crown-6-ether; Dicyclohexano-18-crown-6	14187-32-7	Crown ether	Chemical intermediate

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
1,3-Dibenzoyloxy-17α-ethinyl-7α-methyl-1,3,5(10)-estratrien-17β-ol			Steroid, nonphenolic	
1,3-Dibenzyltetramethyldisiloxane			Siloxane	
Dibutyl benzyl phthalate			Phthalate	
2,6-Di-*tert* -butylphenol	4,4'-Methylenebis; 2,6-Bis(1,1-dimethyl)phenol	128-39-2	Alkylphenol	Chemical intermediate
Dibutyl phthalate	Di-*n* -butyl phthalate ester; Di-*n* -butyl phthalate; Dibutyl 1,2-benzenedicarboxylate	84-74-2	Phthalate	Plasticizer
2,4'-Dichlorobiphenyl	PCB 8	34883-43-7	Polychlorinated biphenyl	Dielectric fluid
2,5-Dichlorobiphenyl	PCB 9	34883-39-1	Polychlorinated biphenyl	Dielectric fluid
3,4-Dichlorobiphenyl	PCB 12	2974-92-7	Polychlorinated biphenyl	Dielectric fluid
3,5-Dichlorobiphenyl	PCB 14	34883-41-5	Polychlorinated biphenyl	Dielectric fluid
4,4'-Dichlorobiphenyl	PCB 15	2050-68-2	Polychlorinated biphenyl	Dielectric fluid
2,5-Dichloro-2'-biphenylol		53905-30-9	Polychlorinated biphenyl	
2,5-Dichloro-3'-biphenylol		53905-29-6	Polychlorinated biphenyl	
2',5'-Dichloro-4-biphenylol	2',5'-Dichloro-4-hydroxybiphenyl	53905-28-5	Polychlorinated biphenyl	
2,6-Dichloro-4'-biphenylol	4-Hydroxyl, 2',6'-dichloro biphenyl	79881-33-7	Polychlorinated biphenyl	
3,4-Dichloro-2'-biphenylol		209613-97-8	Polychlorinated biphenyl	
3,4-Dichloro-3'-biphenylol		14962-34-6	Polychlorinated biphenyl	
3,4-Dichloro-4'-biphenylol		53890-77-0	Polychlorinated biphenyl	
3,5-Dichloro-2'-biphenylol			Polychlorinated biphenyl	
3,5-Dichloro-4'-biphenylol			Polychlorinated biphenyl	
3,5-Dichloro 2-hydroxy-2-methylbut-3-enanalide	M2	16776-82-1	Organochlorine	Pesticide
2,4-Dichlorophenoxyacetic acid	2,4-D	94-75-7	Phenoxy carboxylic acid	Pesticide
2-[[(3,5-Dichlorophenyl)amino]-carbamoyl]oxy]-2-methyl-3-butenoic acid	M1	119209-27-7	Organochlorine	Pesticide
Dieldrin		60-57-1	Organochlorine; Chlorinated cyclodiene	Pesticide
Dienestrol	*trans,trans* -Dienestrol	84-17-3	Diphenylalkene	Pharmaceutical
α-Dienestrol		13029-44-2	Diphenylalkene	
β-Dienestrol		35495-11-5	Diphenylalkene	Pharmaceutical

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
1,3-Diethyl-6,4'-dihydroxy-2-phenylindene			Stilbene	
Di-2-ethylhexyl adipate	Bis(2-ethylhexyl) adipate	103-23-1	Ester	Plasticizer
Diethylhexyl phthalate	Bis(2-ethylhexyl) phthalate	117-81-7	Phthalate	Chemical intermediate
1,3-Diethyl-4-hydroxy-2-phenylindene			Indene	
1,3-Diethyl-6-hydroxy 2-phenylindene			Indene	
meso-p -(α,β-Diethyl-*p* -methylphenethyl)phenol		267408-76-4	Phenol	
Diethyl phthalate	1,2-Benzenedicarboxylic acid, diethyl ester; Diethyl 1,2-benzenedicarboxylate	84-66-2	Phthalate	Solvent; Plasticizer; Pesticide
Diethylstilbestrol		56-53-1	Stilbene	Pharmaceutical
3,3'-Diethylstilbestrol		5959-71-7	Stilbene	
Diethylstilbestrol dimethyl ether	Dimestrol	130-79-0	Stilbene	Pharmaceutical
Diethylstilbestrol epoxide		6052-82-0	Stilbene	
Diethylstilbestrol phenanthrene			Stilbene	
(*rac*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon; Phenol	
5,11-*trans* -Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon; Phenol	
(5*R* ,11*R*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon; Phenol	
(5*S* ,11*S*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon; Phenol	
Dihexyl phthalate		84-75-3	Phthalate	Plasticizer
5,6-Dihydro-8-[2-(dimethylamino)ethoxy]-12-ethyl-11-phenyl-dibenzo[a,e]cyclooctene, hydrate (1:4)		85850-78-8	Triphenylethylene	
Dihydrogenistein	4',5,7-Trihydroxyisoflavan-4-one	21554-71-2	Isoflavone	
Dihydroglycitein	4*H* -1-Benzopyran-4-one, 2,3-dihydro-7-hydroxy-3-(4-hydroxyphenyl)-6-methoxy- (9CI)	94105-88-1	Isoflavone	
5α-Dihydrotestosterone	Androstanolone; Stanolone; 4-Dihydrotestosterone; 4,5α-Dihydrotestosterone	521-18-6	Steroid, nonphenolic	Pharmaceutical
5β-Dihydrotestosterone	17β-Hydroxy-5β-androstan-3-one; Etiocholan-17β-ol-3-one	571-22-2	Steroid, nonphenolic	Pharmaceutical
2,2'-Dihydroxybenzophenone		835-11-0	Benzophenone	
2,4-Dihydroxybenzophenone	Benzoresorcinol	131-56-6	Benzophenone	Chemical additive
4,4'-Dihydroxybenzophenone	Bishydroxy-4-phenylketone	611-99-4	Benzophenone; Phenol	Pharmaceutical; Chemical intermediate (monomer)
4,4'-Dihydroxybiphenyl	4,4'-Biphenol	92-88-6	Biphenyldiol	Chemical intermediate
Dihydroxydiethylstilbestrol		7507-01-9	Stilbene	
6,4'-Dihydroxyflavone		63046-09-3	Flavone	

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
3,3'-Dihydroxyhexestrol		79199-51-2	Diphenolalkane	
2,2'-Dihydroxy-4-methoxybenzophenone	Dioxybenzone	131-53-3	Benzophenone	Chemical additive
3-(2,3 Dihydroxypropoxy)-10-ethyl-11-phenyldibenz[*b,f*]oxepin		85850-89-1	Triphenylethylene	
Diisobutyl phthalate	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester; Phthalic acid, diisobutyl ester	84-69-5	Phthalate	Plasticizer
Diisodecyl phthalate		26761-40-0	Phthalate	Plasticizer
Diisoheptyl phthalate		41451-28-9	Phthalate	Plasticizer
Diisononyl phthalate		28553-12-0	Phthalate	Plasticizer
11β-[2-(*N,N* -Dimethylamino)ethoxy]estra-1,3,5(10)-triene-3,17β-diol			Steroid, phenolic	
3-[2-(Dimethylamino)ethoxy]-11-ethyl-12-phenyl)-6*H* -dibenzo[*b,f*]thioctin		85850-79-9	Triphenylethylene	
3-[2-(Dimethylamino)ethoxy]-10-ethyl-11-phenyldibenz[*b,f*]oxepin		85850-76-6	Triphenylethylene	
7-[2-(Dimethylamino)ethoxy]-11-ethyl-10-phenyldibenz[*b,f*]thiepin		85850-77-7	Triphenylethylene	
11β-[3-(*N,N* -Dimethylamino)-propoxy]estra-1,3,5 (10)-triene-3,17β-diol		130043-38-8	Steroid, phenolic	
α,α-Dimethyl-β-ethylallenolic acid		15372-37-9	Polycyclic aromatic hydrocarbon	
2,6-Dimethylhexestrol		334707-28-7	Diphenolalkane	
1,6-Dimethylnaphthalene		575-43-9	Polycyclic aromatic hydrocarbon	
Dimethyl phthalate		131-11-3	Phthalate	Plasticizer; Solvent
α,α-Dimethylstilbestrol	Dimethylstilbesterol; Dimestrol	552-80-7	Stilbene	Pharmaceutical
Dimethyl sulfoxide	DMSO	67-68-5	Sulfoxide	Solvent
5,11-*trans* -Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon	
(5*R* ,11*R*)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon	
(5*S* ,11*S*)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon	
Di-*n* -octyl phthalate		117-84-0	Phthalate	Plasticizer
Diphenolic acid		126-00-1	Diphenolalkane	Chemical intermediate
trans,trans -1,4-Diphenyl-1,3-butadiene	Benzene, 1,1'-(1,3-butadiene-1,4-diyl)bis-	886-65-7	Diphenylalkene	
4-[1,2-(Diphenyl-1-butenyl)]phenol acetate		100808-55-7	Triphenylethylene	
2,3-Diphenylindenone-1			Indene	
4-[1-(Diphenylmethylene)propyl]phenol acetate		82333-68-4	Triphenylethylene	

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
1,3-Diphenyltetramethyldisiloxane		56-33-7	Siloxane	
5,11-*trans* -Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon	
(5*R* ,11*R*)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon	
(5*S* ,11*S*)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon	
4-Dodecylphenol	4-Laurylphenol	104-43-8	Alkylphenol	Solvent
Doisynoestrol	2-Phenanthrenecarboxylic acid, 1-ethyl-1,2,3,4-tetrahydro-7-methoxy 2-methyl-, *cis* -(±)- (9CI)	15372-34-6	Tetrahydrophenanthrene	
Dopamine	4-(Aminoethyl)catechol	51-61-6	Alkylphenol	Pharmaceutical
Droloxifene	3-Hydroxytamoxifen	82413-20-5	Stilbene; Triphenylethylene	Pharmaceutical
Empenthrin		54406-48-3	Pyrethrin; Pyrethroid	Pesticide
α−Endosulfan		959-98-8	Organochlorine	Pesticide
α,β−Endosulfan	Endosulfan	115-29-7	Organochlorine; Chlorinated cyclodiene	Pesticide
β-Endosulfan	Endosulfan 2	33213-65-9	Organochlorine; Chlorinated cyclodiene	Pesticide
16-Epiestriol	1,3,5(10)-Estratrien-3,16β,17β-triol	547-81-9	Steroid, phenolic	Pharmaceutical
17-Epiestriol	1,3,5(10)-Estratrien-3,16α,17α-triol	1228-72-4	Steroid, phenolic	
Epitestosterone	Androst-4-en-3-one, 17-α-hydroxy-	481-30-1	Steroid, nonphenolic	
Equilenin	*d* -Equilenin	517-09-9	Steroid, phenolic	Pharmaceutical
Equilin		474-86-2	Steroid, phenolic	Pharmaceutical
Equol		531-95-3	Isoflavone	Pharmaceutical
Erythro -MEA	Erythro-α-Ethyl-α'-methyl-4,4'-dihydroxybibenzyl	20576-52-7	Bisphenol	
16α-Estradiol	Estratriene-3,16α-diol	1090-04-6	Steroid, phenolic	
17α-Estradiol	Estra-1,3,5(10)-triene-3,17α-diol; Estra-1,3,5(10)-triene-3,17-diol, (17-α)-	57-91-0	Steroid, phenolic	
17β-Estradiol	E2; Estradiol; Estratriene-3,17β-diol; Estra-1,3,5(10)-triene-3,17-diol, (17β)-	50-28-2	Steroid, phenolic	Pharmaceutical
9β-Estradiol			Steroid, phenolic	
Estradiol 17-acetate			Steroid, phenolic	
17β-Estradiol 3-acetate		4245-41-4	Steroid, nonphenolic	
Estradiol benzoate		50-50-0	Steroid, nonphenolic	Pharmaceutical
Estradiol diacetate		3434-88-6	Steroid, nonphenolic	Pharmaceutical
17β-Estradiol 3-methyl ether		1035-77-4	Steroid, nonphenolic	Pharmaceutical
9-Estratetraene-3,17β-diol	Estra-1,3,5(10),9(11)-tetraene-3,17β-diol	791-69-5	Steroid, phenolic	

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
Estra-1,3,5(10),6-tetraen-17-one, 3-hydroxy-			Steroid, phenolic	
Estra-1,3,5(10)-triene-3,17β-diol,14,15α−epoxy-		79581-12-7	Steroid, phenolic	
Estra-1,3,5(10)-triene-3,17β-diol,14β,15β-epoxy-		79645-49-1	Steroid, phenolic	
Estra-1,3,5(10)-triene-3,14,17β-triol		16288-09-8	Steroid, phenolic	
Estratriene-3,6α,17β-triol		1229-24-9	Steroid, phenolic	Pharmaceutical
Estriol	Estratriene-3,16α,17β-triol; Estratriol; E3	50-27-1	Steroid, phenolic	Pharmaceutical
Estrone	Estratriene-3-ol-17-one; E1	53-16-7	Steroid, phenolic	Pharmaceutical
Estrone 3-acetate		901-93-9	Steroid, nonphenolic	Chemical intermediate
Estrone 3-methyl ether		1624-62-0	Steroid, nonphenolic	Chemical intermediate
Estrone-3-sulfate		481-97-0	Steroid, nonphenolic	Chemical intermediate
17α-Ethinyl estradiol	Ethinylestradiol; Ethynylestradiol	57-63-6	Steroid, phenolic	Pharmaceutical
17β-Ethinyl estradiol		4717-38-8	Steroid, phenolic	Pharmaceutical
Ethyl cinnamate		103-36-6	Phenylalkene	Fragrance
3-Ethyl-6,4'-dihydroxy-2-phenylindene			Indene	
2-Ethylhexyl paraben	2-Ethylhexyl 4-hydroxybenzoate	5153-25-3	Paraben	Food additive
4-Ethyl-7-hydroxy-3-(methoxyphenyl)-2*H* -1-benzopyran-2-one		5219-17-0		
3-[(10-Ethyl-11-*p* -hydroxyphenyl)dibenzo[*b,f*]oxepin-3-yl)oxy]-1,2-propanediol, hydrate (4:1)		85850-93-7	Triphenylethylene	
3-[(10-Ethyl-11-*p* -hydroxyphenyl)dibenzo[*b,f*]thiepin-3-yl)oxy]-1,2-propanediol		85850-94-8	Triphenylethylene	
3-[(11-Ethyl-12-(*p* -hydroxyphenyl)-6-*H* -dibenzo[*b,f*]thiocin-3-yl)oxy]-, hydrate1,2-propanediol		85864-54-6	Triphenylethylene	
3-[(6-Ethyl-5-(*p* -hydroxyphenyl)-11,12-dihydrodibenzo[*a,e*]cycloocten-2-yl)oxy]-1,2-propanediol		85850-95-9	Triphenylethylene	
3-Ethyl-4'-hydroxy-2-phenylindene			Indene	
3-Ethyl-6-hydroxy 2-phenylindene			Indene	
3-Ethyl-4'-hydroxy 2-phenylindenone-1			Indene	
3-Ethyl-6-hydroxy 2-phenylindenone-1			Indene	
3-Ethyl-4-(*p* -methoxyphenyl)-2-methyl-3-cyclohexene-1-carboxylic acid		1755-52-8	Carboxylic acid	
Ethyl paraben	Ethyl 4-hydroxybenzoate; Ethyl-*p* -hydroxybenzoate;	120-47-8	Paraben	Preservative (pharmaceuticals); Food additive
2-Ethylphenol	Phenol, *o* -ethyl-; Phlorol	90-00-6	Alkylphenol	
3-Ethylphenol	Phenol, *m* -ethyl-	620-17-7	Alkylphenol	

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
4-Ethylphenol	Phenol, *p* -ethyl-	123-07-9	Phenol; Alkylphenol	Flavor
3-[(10-Ethyl-11-phenyldibenzo-[*b*,*f*]thiepin-3-yl)oxy]-1,2-propanediol, complexed with isopropyl alcohol 2:1		85850-90-4		
3-[(11-Ethyl-12-phenyl-6*H* -dibenzo [*b*,*f*]thioctin-3-yl)oxy]-1,2-propanediol, hydrate (4:1)		85850-92-6		
3-[(6-Ethyl-5-phenyl-11,12-dihydrodibenzo [*a* ,*e*]cycloocten-2-yl)oxy]-1,2-propanediol		85850-91-5		
Eugenol	Phenol, 4-allyl-2-methoxy-	97-53-0	Alkoxyphenol	Pharmaceutical; Cosmetic (fragrance); Chemical intermediate
Fenvalerate	Pydrin; (+)-alpha-Cyano-3-phenoxybenzyl-(+)-alpha-(4-chlorophenyl)isovalerate	51630-58-1	Organochlorine; Diphenyl ether	Pesticide
Fisetin		528-48-3	Flavone	Natural product
Flavanone		17002-31-2	Flavanone	Natural product
Flavone		525-82-6	Flavone	Natural product
Fluoranthene		206-44-0	Polycyclic aromatic hydrocarbon	
Fluorene		86-73-7	Polycyclic aromatic hydrocarbon	Dye
2-Fluoroestratrien-17β-ol		101772-22-9	Steroid, nonphenolic	
4-Fluoroestratrien-17β-ol		96607-54-4	Steroid, nonphenolic	
2-(2-Fluorophenyl)-3-phenyl-6-hydroxyindene			Indene	
Fluorotamoxifen		73617-96-6	Triphenylethylene	
Folic acid		59-30-3	Pteridine	Pharmaceutical
Formononetin	7-Hydroxy-4'-methoxyisoflavone	485-72-3	Isoflavone	Pharmaceutical; Natural product
Furfural	2-Furaldehye; 2-Furancarboxaldehyde	98-01-1	Heterocyclic aromatic aldehyde	Pesticide
Genistein	4',5,7-Trihydroxyisoflavanone	446-72-0	Isoflavone	Pharmaceutical; Natural product
Genistin	Genistein glucoside	529-59-9	Isoflavone	Natural product
Glyceollin		66241-09-6	Isoflavone	Natural product
Glycitein		40957-83-3	Isoflavone	
Glycitin			Isoflavone	
Heptachlor		76-44-8	Organochlorine; Chlorinated cyclodiene	Pesticide
2,2',3,3',4',5,6-Heptachlorobiphenyl	PCB 177	52663-70-4	Polychlorinated biphenyl	Dielectric fluid
2,2',3,3',4,5,6-Heptachlorobiphenyl	PCB 173	68194-16-1	Polychlorinated biphenyl	Dielectric fluid
2,2',3,3',5,5',6-Heptachlorobiphenyl	PCB 178	52663-64-6	Polychlorinated biphenyl	Dielectric fluid

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
2,2',3,4,4',5',6-Heptachlorobiphenyl	PCB 183	52663-69-1	Polychlorinated biphenyl	Dielectric fluid
2,2',3,4,4',6,6'-Heptachlorobiphenyl	PCB 184	74472-48-3	Polychlorinated biphenyl	Dielectric fluid
2,2',3,4',5,5',6-Heptachlorobiphenyl	PCB 187	52663-68-0	Polychlorinated biphenyl	Dielectric fluid
2,2',3,4',5,6,6'-Heptachlorobiphenyl	PCB 188	74487-85-7	Polychlorinated biphenyl	Dielectric fluid
2,3,3',4,4',5,6-Heptachlorobiphenyl	PCB 190	41411-64-7	Polychlorinated biphenyl	Dielectric fluid
2,3,3',4',5,5',6-Heptachlorobiphenyl	PCB 193	69782-91-8	Polychlorinated biphenyl	Dielectric fluid
2,2',3,3',4',5,5'-Heptachloro-4-biphenylol		158076-64-3	Polychlorinated biphenyl	
2,2',3',4,4',5,5'-Heptachloro-3-biphenylol		158076-69-8	Polychlorinated biphenyl	
2,2',3,4',5,5',6-Heptachloro-4-biphenylol		158076-68-7	Polychlorinated biphenyl	
Heptanal	*n* -Heptaldehyde	111-71-7	Aldehyde	Fragrance
4-(Heptyloxy)phenol	4-Heptyloxyphenol	13037-86-0	Alkoxyphenol	
Heptyl 4-paraben	Benzoic acid, 4-hydroxy-, heptyl ester; Heptyl *p* -hydroxybenzoate	1085-12-7	Paraben	Preservative
Hesperetin		520-33-2	Flavanone	Natural product
Hexachlorobenzene		118-74-1	Organochlorine	Chemical intermediate; Pesticide; Plasticizer
2,2',3,3',4,4'-Hexachlorobiphenyl	PCB 128	38380-07-3	Polychlorinated biphenyl	Dielectric fluid
2,2',3,4,4',5'-Hexachlorobiphenyl	PCB 138	35065-28-2	Polychlorinated biphenyl	Dielectric fluid
2,2',3,4,5,6'-Hexachlorobiphenyl	PCB 143	68194-15-0	Polychlorinated biphenyl	Dielectric fluid
2,2',3,4',5',6-Hexachlorobiphenyl	PCB 149	38380-04-0	Polychlorinated biphenyl	Dielectric fluid
2,2',3,5,5',6-Hexachlorobiphenyl	PCB 151	52663-63-5	Polychlorinated biphenyl	Dielectric fluid
2,2',4,4',5,5'-Hexachlorobiphenyl	PCB 153	35065-27-1	Polychlorinated biphenyl	Dielectric fluid
2,2',4,4',6,6'-Hexachlorobiphenyl	PCB 155	33979-03-2	Polychlorinated biphenyl	Dielectric fluid
2,3,3',4,4',6-Hexachlorobiphenyl	PCB 158	74472-42-7	Polychlorinated biphenyl	Dielectric fluid
2,3',4,4',5',6-Hexachlorobiphenyl	PCB 168	59291-65-5	Polychlorinated biphenyl	Dielectric fluid
3,3',4,4',5,5'-Hexachlorobiphenyl	PCB 169	32774-16-6	Polychlorinated biphenyl	Dielectric fluid
2,2',3,3',4',5-Hexachloro-4-biphenylol		158076-62-1	Polychlorinated biphenyl	
2,2',3,4',5,5'-Hexachloro-4-biphenylol		145413-90-7	Polychlorinated biphenyl	

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
2',3,3',4',5,5'-Hexachloro-4-biphenylol		158076-63-2	Polychlorinated biphenyl	
n -Hexanol	Hexyl alcohol; 1-Hexanol	111-27-3	Alcohol	Chemical intermediate
Hexestrol	*meso* -Hexestrol	84-16-2	Diphenolalkane; Bisphenol	Pharmaceutical
DL -Hexestrol		5776-72-7	Diphenolalkane	Pharmaceutical
Hexestrol monomethyl ether		13026-26-1	Alkoxyphenol	
3-Hydroxybenzo[*b*]naphtho[2,1-d]thiophene			Thiophene	
2-Hydroxybenzo[*c*]phenanthrene		22717-94-8	Polycyclic aromatic hydrocarbon	
3-Hydroxybenzo[*b*]phenanthro[2,3-d]thiophene			Thiophene	
4-Hydroxychalcone		20426-12-4	Chalconoid	
4'-Hydroxychalcone		2657-25-2	Chalconoid	
4'-Hydroxychalcone (*cis*- and *trans*-)		38239-52-0	Chalconoid	
2-Hydroxychrysene	2-Chrysenol	65945-06-4	Polycyclic aromatic hydrocarbon	
4'-Hydroxy-2,3-diphenylindenone-1			Triphenylethylene	
6'-Hydroxy-2,3-diphenylindenone-1			Triphenylethylene	
11α-Hydroxyestradiol		1464-61-5	Steroid, phenolic	
11β-Hydroxyestradiol		5444-22-4	Steroid, phenolic	
14β-Hydroxyestradiol		60183-66-6	Steroid, phenolic	
2-Hydroxyestradiol		362-05-0	Steroid, phenolic	
4-Hydroxyestradiol		5976-61-4	Steroid, phenolic	
2-Hydroxyestratrien-17β-ol	Estratriene-2,17β-diol	2259-89-4	Steroid, phenolic	
4-Hydroxyestratrien-17β-ol	Estratriene-4,17β-diol	17592-89-1	Steroid, phenolic	
3-Hydroxyestra-1,3,5(10)-trien-16-one		3601-97-6	Steroid, phenolic	
2-Hydroxyestrone		362-06-1	Steroid, phenolic	Pharmaceutical
2-Hydroxyethyl methacrylate		868-77-9	Acrylate	Chemical intermediate
3'-Hydroxyflavanone		92496-65-6	Flavanone	
4'-Hydroxyflavanone		135413-27-3	Flavanone	
6-Hydroxyflavanone		4250-77-5	Flavanone	
7-Hydroxyflavanone		6515-36-2	Flavanone	
6-Hydroxyflavone		6665-83-4	Flavone	
7-Hydroxyflavone		6665-86-7	Flavone	
Hydroxyflutamide		52806-53-8	Nitrobenzene	Pharmaceutical
2-Hydroxy-4-methoxybenzophenone	Oxybenzone	131-57-7	Benzophenone	Pharmaceutical

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
6-Hydroxy-2'-methoxyflavone		61546-59-6	Flavone	
2-Hydroxy-5-methylchrysene			Polycyclic aromatic hydrocarbon	
8-Hydroxy-5-methylchrysene			Polycyclic aromatic hydrocarbon	
16β-Hydroxy-16-methyl-17β-estradiol 3-methyl ether	16-Methylestra-1,3,5(10)-triene-3,16β,17β-triol 3-methyl ether	3434-79-5	Steroid, nonphenolic	
4-Hydroxytamoxifen	Hydroxytamoxifen	68047-06-3	Triphenylethylene	Pharmaceutical
6-Hydroxytetralin	5,6,7,8-Tetrahydro-2-naphthol	1125-78-6	Polycyclic aromatic hydrocarbon; Phenol	
ICI 164384		98007-99-9	Steroid, phenolic	Pharmaceutical
ICI 182780		129453-61-8	Steroid, phenolic	Pharmaceutical
Imiprothrin		72963-72-5	Pyrethrin; Pyrethroid	Pesticide
Indanestrol		71855-45-3	Indane	
Indanyldiethylstilbestrol			Stilbene	
Indenestrol A		24643-97-8	Stilbene	
(*R*)-Indenestrol A		115217-03-3	Stilbene	
(*rac*)-Indenestrol A		115217-02-2	Stilbene	
(*S*)-Indenestrol A		115217-04-4	Stilbene	
Indenestrol B		38028-27-2	Stilbene	
(*R*)-Indenestrol B			Stilbene	
(*rac*)-Indenestrol B		133830-97-4	Stilbene	
(*S*)-Indenestrol B		115217-06-6	Stilbene	
Indeno[1,2,3-*cd*]pyrene		193-39-5	Polycyclic aromatic hydrocarbon	
Indole[3,2-*b*]carbazole			Nitrogen heterocycle	
16α-Iodoestradiol		71765-94-1	Steroid, phenolic	
(*E*)-17α-Iodovinylestradiol	E-IVE	82123-96-4	Steroid, phenolic	
(*Z*)-17α-Iodovinylestradiol	Z-IVE	177159-09-0	Steroid, phenolic	
Ipriflavone		35212-22-7	Isoflavone	Pharmaceutical
Isoeugenol	Phenol, 2-methoxy-4-propenyl-	97-54-1	Alkoxyphenol	Natural product; Fragrance; Chemical intermediate (flavor and food additive)
Kaempferol	3,4',5,7-Tetrahydroxyflavone; Kaempherol	520-18-3	Flavone	Natural product
Kepone	Chlordecone	143-50-0	Organochlorine	Pesticide
11-Keto-9β-estradiol			Steroid, phenolic	
16-Ketoestradiol		566-75-6	Steroid, phenolic	
6-Ketoestradiol		571-92-6	Steroid, phenolic	

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
Lindane	γ-Hexachlorocyclohexane	58-89-9	Chlorinated cycloalkane; Organochlorine	Pesticide; Pharmaceutical
Melatonin		73-31-4	Aromatic heterocycle	Pharmaceutical
MER-25	Benzeneethanol, α-[4-[2-diethylaminoethoxy]phenyl]-4-methoxy−α-phenyl-	67-98-1	Alcohol	Pharmaceutical
Mestilbol	Diethylstilbestrol monomethyl ether	18839-90-2	Stilbene	Pharmaceutical
Mestranol	17 alpha-ethylestradiol 3-methyl ether	72-33-3	Steroid, nonphenolic	Pharmaceutical
p,p′ - Methoxychlor	(1,1,1-Trichloro-2,2-bis(p-methoxyphenyl)-ethane	72-43-5	Organochlorine	Pesticide
o,p′ -Methoxychlor		30667-99-3	Organochlorine	Pesticide
Methyltamoxifen		73617-95-5	Triphenylethylene	
Methyl paraben	Methyl 4-hydroxybenzoate	99-76-3	Paraben	Preservative (foods and cosmetics); Food additive
Methyl methacrylate		80-62-6	Acrylate	Chemical intermediate
Methoxytamoxifen			Triphenylethylene	
Methoxychlor olefin		2132-70-9	Organochlorine	Pesticide
9α-Methylestrone 3-methyl ether		31266-41-8	Steroid, nonphenolic	
9α-Methylestrone		71563-77-4	Steroid, phenolic	
9α-Methylestradiol-17β 3-methyl ether		51242-32-1	Steroid, nonphenolic	
9α-Methylestradiol-17β		66463-44-3	Steroid, phenolic	
9α-Methyl-14-dehydroestrone 3-methyl ether			Steroid, nonphenolic	
9α-Methyl-14-dehydroestrone		88598-67-8	Steroid, phenolic	
9α-Methyl-14-dehydroestradiol-17β 3-methyl ether		88598-64-5	Steroid, nonphenolic	
9α-Methyl-14-dehydroestradiol-17β		88598-63-4	Steroid, phenolic	
7α-Methylestrone 3-methyl ether		10449-00-0	Steroid, nonphenolic	
7α−Methylestrone		10448-96-1	Steroid, phenolic	Pharmaceutical
7α-Methylestradiol-17β 3-methyl ether		15506-01-1	Steroid, nonphenolic	
7α-Methylestradiol-17β		10448-97-2	Steroid, phenolic	
7α-Methyl-14-dehydroestrone 3-methyl ether		35644-57-6	Steroid, nonphenolic	
7α-Methyl-14-dehydroestrone		88958-66-7	Steroid, phenolic	
7α-Methyl-14-dehydroestradiol-17β 3-methyl ether		35644-59-8	Steroid, nonphenolic	
7α-Methyl-14-dehydroestradiol-17β		88598-62-3	Steroid, phenolic	
4,4'-Methylenedianiline	Benzenamine, 4,4'-methylenebis- ; 4,4'-Methylenebis(aniline); 4,4'-Methylenebisbenzenamine	101-77-9	Aniline	Chemical intermediate

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
4,4'-Methylenebis(N,N-dimethylaniline)	4,4'-Methylenebis(N,N-dimethylbenzenamine); Michler's base	101-61-1	Aniline	Chemical intermediate
3-Methoxyestriol	Estriol 3-methyl ether	1474-53-9	Steroid, nonphenolic	
3-Methoxy-10-methyl-11-phenyldibenzo[*b,f*] thiepin (16β)		85807-06-1	Triphenylethylene	
2-(2-Methylphenyl)-3-phenyl-6-hydroxyindene			Triphenylethylene	
1-Methyl-6-hydroxy-2,3-diphenylindene			Triphenylethylene	
1-Methyl-3-ethyl-6,4'-dihydroxy-2-phenylindene			Stilbene	
11β-Methylestrone 3-methyl ether		13667-04-4	Steroid, nonphenolic	
11β-Methylestrone		13667-06-6	Steroid, phenolic	
11β-Methylestradiol-17β 3-methyl ether		18046-75-8	Steroid, nonphenolic	
11β-Methylestradiol-17β	11β-Methylestradiol	23637-93-6	Steroid, phenolic	
11β-Methyl-14-dehydroestrone 3-methyl ether		88598-69-0	Steroid, nonphenolic	
11β-Methyl-14-dehydroestradiol-17β 3-methyl ether		88598-65-6	Steroid, nonphenolic	
(*Z*)-11β-Methoxy-17α-iodovinylestradiol	Z-MIVE	177159-11-4	Steroid, phenolic	
(*E*)-11β-Methoxy-17α-iodovinylestradiol	E-MIVE	90857-55-9	Steroid, phenolic	
Metolachlor		51218-45-2	Acetamide; Anilide	Pesticide
Mirex	Dechlorane	2385-85-5	Organochlorine	Pesticide; Fire retardant for plastics, rubber, paint
Mono-*m* -acetoxy-1,1,2-triphenylbut-1-ene		82333-69-5	Triphenylethylene	
Monohydroxymethoxychlor		28463-03-8	Organochlorine	Pesticide metabolite
Monohydroxymethoxychlor olefin		75938-34-0	Organochlorine	Pesticide
Monohydroxytamoxifen		68392-35-8	Triphenylethylene	
Morin		480-16-0	Flavone	Dye
Moxestrol	R-2358, 11β-Methoxy-17α-ethinylestradiol	34816-55-2	Steroid, phenolic	Pharmaceutical
Myricetin		529-44-2	Flavone	Natural product
Nafoxidine	Pyrrolidine, 1-(2-(4-(3,4-dihydro-6-methoxy-2-phenyl-1-naphthalenyl)phenoxy)ethyl)-	1845-11-0	Triphenylethylene; Stilbene	Pharmaceutical
Naringenin	4,5,7-Trihydroxyflavanone 7-rhamnoglucoside	480-41-1	Flavanone	
Naringin		10236-47-2	Flavanone	Food additive
Nerolidol		7212-44-4	Terpene	Fragrance
2-Nitroestratriene-3,17β-diol	2-Nitro-1,3,5(10)-estratriene-3,17β-diol	6298-51-7	Steroid, phenolic	

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
4-Nitroestratriene-3,17β-diol	4-Nitro-1,3,5(10)-estratriene-3,17β-diol; 4-Nitroestradiol	6936-94-3	Steroid, phenolic	
2-Nitroestratrien-3-ol-17-one	2-Nitroestrone	5976-73-8	Steroid, phenolic	
4-Nitroestratrien-3-ol-17-one	4-Nitroestrone	5976-74-9	Steroid, phenolic	
Nitromifene	CN-55,945	10448-84-7	Triphenylethylene	Pharmaceutical
cis -Nonachlor		5103-73-1	Organochlorine; Chlorinated bridged cycloalkene	Pesticide
trans -Nonachlor		39765-80-5	Organochlorine; Chlorinated bridged cycloalkene	Pesticide
Nonylbenzene		1081-77-2	Aromatic hydrocarbon	
p -Nonylphenol	*p-n* -Nonylphenol; 4-Nonylphenol	104-40-5	Phenol; Alkylphenol	Chemical intermediate
n -Nonylphenol		25154-52-3	Phenol; Alkylphenol	Chemical intermediate
Nonylphenol dodecylethoxylate			Phenol; Alkylphenol	Surfactant
Nordihydroguariaretic acid		500-38-9	Bisphenol	Pharmaceutical
Norethindrone		68-22-4	Steroid, nonphenolic	Pharmaceutical
Norethynodrel		68-23-5	Steroid, nonphenolic	Pharmaceutical
19-Nortestosterone		434-22-0	Steroid, nonphenolic	Pharmaceutical
2,2',3,3',4,4',5,5'-Octachlorobiphenyl		35694-08-7	Polychlorinated biphenyl	Dielectric fluid
1,8-Octanediol	1,8-Octamethylenediol	629-41-4	Alcohol	Pharmaceutical
4-*n* -Octylphenol	4-Octylphenol; *p* -Octylphenol	1806-26-4	Alkylphenol	Chemical intermediate
4-*tert* -Octylphenol	*p-tert*- Octylphenol; 4-(1,1,3,3-Tetramethylbutyl)phenol	140-66-9	Alkylphenol	Chemical intermediate
2,2',3,3',6-Pentachlorobiphenyl	PCB 84	52663-60-2	Polychlorinated biphenyl	Dielectric fluid
2,2',3,4,5'-Pentachlorobiphenyl	PCB 87	38380-02-8	Polychlorinated biphenyl	Dielectric fluid
2,2',3,4',6-Pentachlorobiphenyl	PCB 91	68194-05-8	Polychlorinated biphenyl	Dielectric fluid
2,2',3,5',6-Pentachlorobiphenyl	PCB 95	38379-99-6	Polychlorinated biphenyl	Dielectric fluid
2,2',4,4',5-Pentachlorobiphenyl	PCB 99	38380-01-7	Polychlorinated biphenyl	Dielectric fluid
2,2',4,5,5'-Pentachlorobiphenyl	PCB 101	37680-73-2	Polychlorinated biphenyl	Dielectric fluid
2,2',4,6,6'-Pentachlorobiphenyl	PCB 104	56558-16-8	Polychlorinated biphenyl	Dielectric fluid
2,3,3',5,6-Pentachlorobiphenyl	PCB 112	74472-36-9	Polychlorinated biphenyl	Dielectric fluid
2,3,4,4',6-Pentachlorobiphenyl	PCB 115	74472-38-1	Polychlorinated biphenyl	Dielectric fluid
3,3',4,4',5-Pentachlorobiphenyl	PCB 126	57465-28-8	Polychlorinated biphenyl	Dielectric fluid

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
2,2',3',4',5'-Pentachloro-4-biphenylol		150304-12-4	Polychlorinated biphenyl	
2,2',3',4',6'-Pentachloro-4-biphenylol		150304-10-2	Polychlorinated biphenyl	
2,2',3',5',6'-Pentachloro-4-biphenylol		150304-11-3	Polychlorinated biphenyl	
2,2',4,6,6'-Pentachloro-4-biphenylol			Polychlorinated biphenyl	
2',3,3',4,4'-Pentachloro-2-biphenylol		150975-80-7	Polychlorinated biphenyl	
2,3,3',4',5-Pentachloro-4-biphenylol		152969-11-4	Polychlorinated biphenyl	
2',3,3',4',5-Pentachloro-4-biphenylol		192190-09-3	Polychlorinated biphenyl	
2',3,3',4',5'-Pentachloro-4-biphenylol		149589-55-9	Polychlorinated biphenyl	
2',3,3',4',6'-Pentachloro-4-biphenylol		192190-10-6	Polychlorinated biphenyl	
2',3,3',5',6'-Pentachloro-4-biphenylol		189578-02-7	Polychlorinated biphenyl	
2',3',4,4',5-Pentachloro-3-biphenylol		150975-81-8	Polychlorinated biphenyl	
2,3',4,4',5-Pentachloro-3-biphenylol		170946-11-9	Polychlorinated biphenyl	
2',3,4',5,5'-Pentachloro-4-biphenylol		149589-56-0	Polychlorinated biphenyl	
3,3',4',5,5'-Pentachloro-4-biphenylol		130689-92-8	Polychlorinated biphenyl	
Pentolame		150748-24-6	Steroid, phenolic	
Permethrin	(3-Phenoxyphenyl)methyl (+-) *cis,trans* -3-(2,2-dichloroethenyl)-2,2-dimethyl cyclopropane-carboxylate	52645-53-1	Pyrethrin; Pyrethroid	Pesticide
Phenanthrene		85-01-8	Polycyclic aromatic hydrocarbon	Chemical intermediate
4-Phenethylphenol		6335-83-7	Alkylphenol	
Phenol, 4,4'-[1,2-bis(methylene)-1,2-ethanediyl]bis-		107144-81-0	Bisphenol	Chemical intermediate
Phenol, 4-[7-(2-dimethylamino)ethoxy]-11-ethyldibenzo[*b,f*]thiepin-10-yl]-	3-[2-(Dimethylamino)ethoxy]-10-ethyl-11-(4-hydroxyphenyl)-dibenzo[*b,f*]thiepin ethyl acetate	85850-74-4	Triphenylethylene	
Phenol, 4-[3-(2 dimethylamino)ethoxy]-11-ethyldibenzo[*b,f*]thioctin-12-yl)	Phenol, *p* -[3-(2-(dimethylamino)-ethoxy]-11-ethyl-6*H* -dibenzo-[*b,f*]thiocin-12-yl)-hydrate	85850-81-3	Triphenylethylene	
Phenol, 4-[2-(2 dimethylamino)-ethoxy]-6-ethyl-11,12-dihydro-dibenzo[*a,e*]cycloocten-5-yl]-		85850-75-5	Triphenylethylene	
Phenol, 3-[2-dimethylaminoethoxy]-10-ethyl- 4-hydroxyphenyl dibenzo-[*b,f*]oxepin	3-[2-(Dimethylamino)ethoxy]-10-ethyl-(4-hydroxyphenyl)-dibenzo[*b,f*]oxepin	85850-80-2	Triphenylethylene	

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
Phenol, 4-[1-[4-[2-(dimethylamino)ethoxy]phenyl]-2-phenyl-1-butenyl]-3-methyl-, (*E*)-	2-Methyl-4-hydroxytamoxifen	96474-35-0	Triphenylethylene	
Phenol, 4-(1, 2-diphenyl-1-butenyl)-		69967-79-9	Triphenylethylene	
Phenol, 4-(1*Z*)-1,2-diphenyl-1-butenyl)-		69967-80-2	Triphenylethylene	
Phenol, 4-[2-Nitro-2-phenyl-1-[4-[2-(1 pyrrolidinyl)ethoxy]phenyl]ethenyl] phenyl, (*E*)-		107144-84-3	Triphenylethylene	
Phenol, 4,4'-(2-phenyl-1-butenylidene)bis-		91221-46-4	Triphenylethylene	
Phenolphthalein		77-09-8	Triphenylmethane	Pharmaceutical
Phenolphthalin		81-90-3	Triphenylmethane	Analytical reagent
Phenol Red	Phenolsulfonphthalein; Phenol, 4,4'-(3*H* -2,1-benzoxathiol-3-ylidene)di-, *S,S* -dioxide	143-74-8	Diphenolalkane; Bisphenol	Pharmaceutical
d -Phenothrin		26002-80-2	Pyrethrin; Pyrethroid	Pesticide
2-Phenyl-3-(2-fluoro-4-hydroxyphenyl)-6-hydroxyindene			Indene	
2-Phenyl-3-(2-fluorophenyl)-6-hydroxyindene			Indene	
3-Phenyl-4'-hydroxy-2-phenylindene			Indene	
3-Phenyl-6-hydroxy-2-phenylindene			Indene	
2-Phenyl-3-(2-methylphenyl)-6-hydroxyindene			Indene	
2-Phenyl-3-(4-methylphenyl)-6-hydroxyindene			Indene	
2-Phenylphenol	*o* -Phenylphenol; 2-Hydroxydiphenyl	90-43-7	Biphenyl; Phenol	Pesticide; Chemical intermediate; Plasticizer; Polymer
3-Phenylphenol		580-51-8	Biphenyl; Phenol	
4-Phenylphenol	*p* -Phenylphenol; 4-Hydroxybiphenyl	92-69-3	Biphenyl; Phenol	Pesticide; Chemical intermediate; Polymer
Phloretin		60-82-2	Chalconoid	Natural product
Prallethrin		23031-36-9	Pyrethrin; Pyrethroid	Pesticide
Progesterone	Pregn-4-ene-3,20-dione	57-83-0	Steroid, nonphenolic	Pharmaceutical
Prolame		99876-41-2	Steroid, phenolic	
Promegesterone	R5020; 17,21-Dimethyl-19-nor-4,9 pregnadiene-3,20-dione	34184-77-5	Steroid, nonphenolic	Pharmaceutical
Prometon	Prometone; Pramitol; Gesafram 50®; Ontracic800®	1610-18-0	Triazine	Pesticide
Propazine	2-Chloro-4,6-bis(isopropylamino)-s-triazine	139-40-2	Triazine	Pesticide
Propyl paraben	Propyl 4-hydroxybenzoate; Benzoic acid, *p* -hydroxy-, propyl ester; Benzoic acid, 4-hydroxy-, propyl ester	94-13-3	Paraben	Pharmaceutical; Preservative (foods); Food additive
Propylpyrazoletriol			Pyrazole	

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
Prunetin		552-59-0	Isoflavone	Natural product
Pseudodiethylstilbestrol	Pseudo-DES	39011-86-4	Bisphenol; Stilbene	
Pyrene		129-00-0	Polycyclic aromatic hydrocarbon	Dye
Pyrrolidine, 1-[2-[4-[1-(4-methoxyphenyl)-2-nitro-2-phenylethenyl]phenoxy]ethyl]-, (*E*)		77413-87-7	Triphenylethylene	
Quercetin	3,3'4',5,7-Pentahydroxyflavone	117-39-5	Flavone	Natural product (plant)
7-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-		107144-83-2		
6-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-		107144-82-1		
Raloxifene		84449-90-1	Stilbene; Piperidine; Phenol	Pharmaceutical
Raloxifene hydrochloride	LY 156758	82640-04-8	Nitrogen heterocycle	Pharmaceutical
Resveratrol	3,4',5-Stilbenetriol	501-36-0	Stilbene	Natural product
Rutin		153-18-4	Flavone	Natural product
Simazine	s-Triazine, 2-chloro-4,6-bis(ethylamino)- ; 1,3,5-Triazine-2,4-diamine, 6-chloro-N,N'-diethyl-	122-34-9	Organochlorine; Triazine	Pesticide
β-Sitosterol	24-alpha-Ethylcholesterol	83-46-5	Steroid, nonphenolic	Natural product (animal); Pharmaceutical
4,4'-Stilbenediol	4,4'-Dihydroxystilbene; Phenol, 4,4'-(1,2-ethenediyl)bis-	659-22-3	Stilbene; Bisphenol	
4-Stilbenol		3839-46-1	Stilbene; Phenol	
Suberic acid	Octanedioic acid	505-48-6	Carboxylic acid	Chemical intermediate
Tamoxifen	ICI 47699	10540-29-1	Stilbene	Pharmaceutical
Tamoxifen citrate		54965-24-1	Stilbene	Pharmaceutical
Taxifolin		480-18-2	Flavanone	Pharmaceutical
Testosterone	Androst-4-en-3-one, 17-hydroxy-, (17β)-	58-22-0	Steroid, nonphenolic	Pharmaceutical
2,2',3,3'-Tetrachlorobiphenyl	PCB 44	3844-93-8	Polychlorinated biphenyl	Dielectric fluid
2,2',3,4-Tetrachlorobiphenyl	PCB 41	52663-59-9	Polychlorinated biphenyl	Dielectric fluid
2,2',3,6-Tetrachlorobiphenyl	PCB 45	41464-47-5	Polychlorinated biphenyl	Dielectric fluid
2,2',4,4'-Tetrachlorobiphenyl	PCB 47	2437-79-8	Polychlorinated biphenyl	Dielectric fluid
2,2',4,5'-Tetrachlorobiphenyl	PCB 49	41464-40-8	Polychlorinated biphenyl	Dielectric fluid
2,2',4,6'-Tetrachlorobiphenyl	PCB 51	68194-04-7	Polychlorinated biphenyl	Dielectric fluid
2,2',5,5'-Tetrachlorobiphenyl	PCB 52	35693-99-3	Polychlorinated biphenyl	Dielectric fluid

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
2,2',6,6'-Tetrachlorobiphenyl	PCB 54	15968-05-5	Polychlorinated biphenyl	Dielectric fluid
2,3,3',5'-Tetrachlorobiphenyl	PCB 58	41464-49-7	Polychlorinated biphenyl	Dielectric fluid
2,3,4,4'-Tetrachlorobiphenyl	PCB 60	33025-41-1	Polychlorinated biphenyl	Dielectric fluid
2,3',4,5'-Tetrachlorobiphenyl	PCB 68	73575-52-7	Polychlorinated biphenyl	Dielectric fluid
2,3',4',5-Tetrachlorobiphenyl	PCB 70	32598-11-1	Polychlorinated biphenyl	Dielectric fluid
2,4,4',5-Tetrachlorobiphenyl	PCB 74	32690-93-0	Polychlorinated biphenyl	Dielectric fluid
3,3',4,4'-Tetrachlorobiphenyl	PCB 77	32598-13-3	Polychlorinated biphenyl	Dielectric fluid
3,3',4,5-Tetrachlorobiphenyl	PCB 78	70362-49-1	Polychlorinated biphenyl	Dielectric fluid
2',3',5',6'-Tetrachloro-4,4'-biphenyldiol		100702-98-5	Polychlorinated biphenyl	
3,3',5,5'-Tetrachloro-4,4'-biphenyldiol		13049-13-3	Polychlorinated biphenyl	
2,2',4',6'-Tetrachloro-4-biphenylol		150304-08-8	Polychlorinated biphenyl	
2,2',6,6'-Tetrachloro-4-biphenylol		219952-18-8	Polychlorinated biphenyl	
2',3',4',5'-Tetrachloro-3-biphenylol		67651-37-0	Polychlorinated biphenyl	
2',3',4',5'-Tetrachloro-4-biphenylol		67651-34-7	Polychlorinated biphenyl	
2',3,4',6'-Tetrachloro-4-biphenylol		189578-00-5	Polychlorinated biphenyl	
2,3,7,8-Tetrachlorodibenzo-p-dioxin	Dioxin; 2,3,7,8-TCDD	1746-01-6	Dioxin	
Tetrahydrochrysene		104460-72-2	Polycyclic aromatic hydrocarbon	
(R,R) -Tetrahydrochrysene			Polycyclic aromatic hydrocarbon	
(S,S) -Tetrahydrochrysene			Polycyclic aromatic hydrocarbon	
2,2',4,4'-Tetrahydroxybenzil		5394-98-9	Benzophenone	
Tetramethylhexestrol		74385-27-6	Stilbene	
Thalidomide		50-35-1	Phthalimide	Pharmaceutical
Toremifene citrate		89778-27-8	Triphenylethylene	Pharmaceutical
Tosyl nonylphenol (mixed branched isomers)			Phenol; Alkylphenol	
Toxaphene		8001-35-2	Organochlorine	Pesticide
Triaryl-pyrazole				
2,2',5-Trichlorobiphenyl	PCB 18	37680-65-2	Polychlorinated biphenyl	Dielectric fluid

Information Sorted by Substance Name

Substance	Synonyms	CASRN	Chemical Class	Product Class
2,4,6-Trichlorobiphenyl	PCB 30	35693-92-6	Polychlorinated biphenyl	Dielectric fluid
2',4',6'-Trichloro-4-biphenylol		14962-28-8	Polychlorinated biphenyl	
3,3',4-Trichloro-4-biphenylol		124882-64-0	Polychlorinated biphenyl	
3,4',5-Trichloro-4-biphenylol		4400-06-0	Polychlorinated biphenyl	
2,4,5-Trichlorophenoxyacetic acid	2,4,5-T acid; Esterone 245; Trioxone; Trichlorophenoxyacetic acid	93-76-5	Chlorinated aromatic hydrocarbon; Organochlorine	Pesticide
Triethylamine, 2-[*p* -[6-methoxy-2-phenyl-3-inden-3-yl)phenoxy] hydrochloride	U-11555A	64-96-0	Triphenylethylene	
Triethylene glycol dimethacrylate		109-16-0	Acrylate	Plasticizer
4,2',4'-Trihydroxychalcone		961-29-5	Chalconoid	
3,6,4',-Trihydroxyflavone		253195-19-6	Flavone	
6,7,4'-Trihydroxyisoflavone		17817-31-1	Isoflavone	
7,3',4'-Trihydroxyisoflavone		485-63-2	Isoflavone	
1,1,2-Triphenylbut-1-ene		63019-13-6	Triphenylethylene	
Triphenylethylene		58-72-0	Triphenylethylene	
Triphenyl phosphate	Phosphoric acid, triphenyl ester	115-86-6	Phosphate ester	Plasticizer
Tris(4-chlorophenyl)methane	Tris-H	27575-78-6	Organochlorine	
Tris(4-chlorophenyl)methanol	Tris-OH	30100-80-8	Organochlorine	
Vanillin	4-Hydroxy-3-methoxybenzaldehyde	121-33-5	Alkoxyphenol	Flavor; Chemical intermediate; Pharmaceutical (additive); Food additive
Vinclozolin		50471-44-8	Organochlorine	Pesticide
α−Zearalanol		26538-44-3	Resorcylic acid lactone; Phenol	Natural product
β−Zearalanol		42422-68-4	Resorcylic acid lactone	Natural product
Zearalanone		5975-78-0	Resorcylic acid lactone	Natural product
α-Zearalenol		36455-72-8	Resorcylic acid lactone; Phenol	Natural product
Zearalenone		17924-92-4	Resorcylic acid lactone; Phenol	Natural product
β−Zearalenol		71030-11-0	Resorcylic acid lactone; Phenol	Natural product

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Appendix D

Substances Tested in the *In Vitro* ER Binding Assays

D1 Data Sorted by Substance Name and Assay

D2 References

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Appendix D1

Substances Tested in the *In Vitro* ER Binding Assays

Data Sorted by Substance Name and Assay

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Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (μM)**	SD of IC_{50}**	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
hERα-FP	4,4'-(1,3-Adamantanediyl)diphenol			0.2	0.001			6.5	0.813	Nikov et al. (2001)
hERα-FP	2-(1-Adamantyl)-4-methylphenol	41031-50-9				10				Nikov et al. (2001)
hERα-FP	4-(1-Adamantyl)phenol	29799-07-3		1	1			1.3	0.114	Nikov et al. (2001)
hERα	Alachlor	15972-60-8				50				Klotz et al. (1996)
RUC	Alachlor	15972-60-8	98.8			100				Blair et al. (2000)
RUC	Aldosterone	52-39-1	98			100				Blair et al. (2000)
RUC	Aldrin	309-00-2	98			600				Blair et al. (2000)
hERα-FP	Allethrin	584-79-2	> 93			10				Saito et al. (2000)
RUC	*p* -(7-Alloxyl)-11-ethyldibenzo-[*b,f*]thiepin-10-yl)phenol	85850-86-8						5.2	0.716	Acton et al. (1983)
RUC	*p* -(3-(Alloxyl)-11-ethyl-6*H* -dibenzo[*b,f*]thiocin-12-yl)phenol hemihydrate	85850-88-0						9.2	0.964	Acton et al. (1983)
RUC	*p* -(2-(Alloxyl)-6-ethyl-11,12-dihydroxydibenzo[*a,e*]cyclooctene-5-yl)phenol	85850-87-9						15	1.176	Acton et al. (1983)
RUC	3-(Alloxyl)-10-ethyl-11-(4-hydroxyphenyl)dibenzo[*b,f*]thiepin	85850-85-7						0.21	-0.678	Acton et al. (1983)
RUC	3-(Alloxyl)-10-ethyl-11-phenyldibenzo[*b,f*]thiepin	85850-82-4						0.54	-0.268	Acton et al. (1983)
RUC	3-(Alloxyl)-11-ethyl-12-phenyl 6*H* -dibenzo[*b,f*]thiocin	85850-84-6						0.12	-0.921	Acton et al. (1983)
RUC	3- (Alloxyl)-10-ethyl-11-phenyldibenz[*b,f*]oxepin	83807-07-2						0.1	-1.000	Acton et al. (1983)
RUC	3-(Alloxyl)-11-ethyl-12-phenyl 5,6-dihydroxydibenzo- [*a,e*]cyclooctene	85850-83-5						0.36	-0.444	Acton et al. (1983)
RUC	Amaranth	915-67-3	80			100				Blair et al. (2000)
MCF-7 cytosol	2-Aminoestratriene-3,17β-diol	107900-30-1						12	1.079	Brooks et al. (1987)
MCF-7 cytosol	4-Aminoestratriene-3,17β-diol	107900-31-2						16	1.204	Brooks et al. (1987)
MCF-7 cytosol	2-Aminoestratrien-17β-ol	17522-06-4						4	0.602	Brooks et al. (1987)
MCF-7 cytosol	4-Aminoestratrien-17β-ol	17522-04-2						0.17	-0.770	Brooks et al. (1987)
RUC	4-Aminophenyl ether	101-80-4	99			1000				Blair et al. (2000)
hERα	4-*tert* -Amylphenol	80-46-6				10				Kuiper et al. (1998) [method a]
hERβ	4-*tert* -Amylphenol	80-46-6				10				Kuiper et al. (1998) [method a]
RUC	4-*tert* -Amylphenol	80-46-6	99	165	45			0.0005	-3.260	Blair et al. (2000)
hERα	3β-Androstanediol	25126-76-5					0.006	3	0.477	Kuiper et al. (1997)
rERβ	3β-Androstanediol	25126-76-5					0.002	7	0.845	Kuiper et al. (1997)
MUC	5α-Androstane-3α,17β-diol	1852-53-5				10				Korach (1979)
RUC	5α-Androstane-3α,17β-diol	1852-53-5	99	42	1.6			0.002	-2.670	Blair et al. (2000)
MCF-7 cytosol	5α-Androstane-3α,17β-diol	1852-53-5				0.1				VanderKuur et al. (1993)
hERα	5α-Androstane-3β,17β-diol	571-20-0					0.26	0.07	-1.150	Kuiper et al. (1997)
MUC	5α-Androstane-3β,17β-diol	571-20-0						0.5	-0.300	Korach (1979)
rERβ	5α-Androstane-3β,17β-diol	571-20-0					0.048	0.3	-0.523	Kuiper et al. (1997)
MCF-7 cytosol	5α-Androstane-3β,17β-diol	571-20-0						0.005	-2.301	VanderKuur et al. (1993)
RUC	5α-Androstane-3β,17β-diol	571-20-0		0.75	0.13			0.12	-0.920	Blair et al. (2000)
MUC	5β-Androstane-3α,17β-diol	1851-23-6				10				Korach (1979)
hERα	5β-Androstanedione	1229-12-5				100				Kuiper et al. (1997)
rERβ	5β-Androstanedione	1229-12-5				100				Kuiper et al. (1997)
hERα	5β-Androstane-3,17-dione	5982-99-0				100				Kuiper et al. (1997)
MUC	5β-Androstane-3,17-dione	5982-99-0				10				Korach (1979)
rERβ	5β-Androstane-3,17-dione	5982-99-0				100				Kuiper et al. (1997)
MUC	5α-Androstane-3α-ol-17-one	53-41-8				10				Korach (1979)
hERα	4-Androstenediol	1156-92-9					0.023	0.5	-0.300	Kuiper et al. (1997)
rERβ	4-Androstenediol	1156-92-9					0.019	0.6	-0.222	Kuiper et al. (1997)
hERα	5-Androstenediol	521-17-5					0.0036	6	0.778	Kuiper et al. (1997)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (µM)**	SD of IC_{50}**	HDT (µM)	Ki (µM)**	RBA***	log RBA***	Reference
hERα	5-Androstenediol	521-17-5						1	0.000	Kuiper et al. (1998) [method a]
hERα	5-Androstenediol	521-17-5		0.024				3.9	0.590	Kuiper et al. (1998) [method b]
hERβ	5-Androstenediol	521-17-5						7	0.845	Kuiper et al. (1998) [method a]
hERβ	5-Androstenediol	521-17-5		0.005				21.2	1.330	Kuiper et al. (1998) [method b]
RBC	5-Androstenediol	521-17-5						1	0.000	Korenman (1969)
rERβ	5-Androstenediol	521-17-5					0.0009	17	1.230	Kuiper et al. (1997)
hERα	4-Androstenedione	63-05-8				100				Kuiper et al. (1997)
MCF-7 cytosol	4-Androstenedione	63-05-8						0.007	-2.155	VanderKuur et al. (1993)
MUC	4-Androstenedione	63-05-8				10				Korach (1979)
rERβ	4-Androstenedione	63-05-8				100				Kuiper et al. (1997)
MCF-7 cells	Anthracene	120-12-7				5				Arcaro et al. (1999)
hERα	Apigenin	520-36-5						0.3	-0.523	Kuiper et al. (1998) [method a]
hERβ	Apigenin	520-36-5						6	0.778	Kuiper et al. (1998) [method a]
hERβ	Apigenin	520-36-5		0.058				2	0.301	Kuiper et al. (1998) [method b]
RUC	Apigenin	520-36-5						0.028	-0.620	Fang et al. (2001)
RUC	Aroclor 1221	11104-28-2				*100* [a]				Nelson (1974)
RUC	Aroclor 1254	11097-69-1				*100*				Nelson (1974)
GST-aERdef	Atrazine	1912-24-9				100				Matthews et al. (2000)
GST-cERdef	Atrazine	1912-24-9				100				Matthews et al. (2000)
GST-hERαdef	Atrazine	1912-24-9				100				Matthews et al. (2000)
GST-mERαdef	Atrazine	1912-24-9				100				Matthews et al. (2000)
GST-rtERdef	Atrazine	1912-24-9				100				Matthews et al. (2000)
hERα-FP	Atrazine	1912-24-9	99.1			2000				Hanioka et al. (1999)
RUC	Atrazine	1912-24-9	98			100				Blair et al. (2000)
RUC	Atrazine	1912-24-9					1000	0.0003	-3.523	Waller et al. (1996)
RUC	Aurin	603-45-2		2.8	1.8			0.032	-1.490	Blair et al. (2000)
RUC	Baicalein	491-67-8						0.0009	-3.046	Fang et al. (2001)
hERα	Benomyl	17804-35-2				50				Klotz et al. (1996)
GST-hERαdef	Benz[*a*]anthracene	56-55-3				10				Fertuck et al. (2001)
hERβ	Benz[*a*]anthracene	56-55-3				10				Fertuck et al. (2001)
MCF-7 cells	Benz[*a*]anthracene	56-55-3						33	1.519	Arcaro et al. (1999)
MCF-7 cells	Benzeneacetonitrile α-[bis(4-hydroxyphenyl) methylene]	66422-14-8						8.5	0.929	Stoessel and Leclerq (1986)
MCF-7 cytosol	Benzeneacetonitrile α-[bis(4-hydroxyphenyl) methylene]	66422-14-8						100	2.000	Stoessel and Leclerq (1986)
GST-hERαdef	Benzo[*a*]carbazole	239-01-0				10				Fertuck et al. (2001)
hERβ	Benzo[*a*]carbazole	239-01-0				10				Fertuck et al. (2001)
GST-hERαdef	Benzo[*c*]carbazole					10				Fertuck et al. (2001)
hERβ	Benzo[*c*]carbazole					10				Fertuck et al. (2001)
MCF-7 cells	Benzo[*b*]fluoranthene	205-99-2						17	1.230	Arcaro et al. (1999)
MCF-7 cells	Benzo[*k*]fluoranthene	207-08-9						27	1.431	Arcaro et al. (1999)
RUC	Benzo[*a*]fluorene	238-84-6	98			33.3				Blair et al. (2000)
GST-hERαdef	Benzo[*b*]fluorene	243-17-4				10				Fertuck et al. (2001)
hERβ	Benzo[*b*]fluorene	243-17-4				10				Fertuck et al. (2001)
GST-hERαdef	Benzo[*b*]naptho[2,1-d]thiophene	239-35-0				10				Fertuck et al. (2001)
hERβ	Benzo[*b*]naptho[2,1-d]thiophene	239-35-0				10				Fertuck et al. (2001)
GST-hERαdef	Benzo[*b*]naptho[2,3-d]thiophene	243-46-9				10				Fertuck et al. (2001)
hERβ	Benzo[*b*]naptho[2,3-d]thiophene	243-46-9				10				Fertuck et al. (2001)
MCF-7 cells	Benzo[*ghi*]perylene	191-24-2				5				Arcaro et al. (1999)
GST-hERαdef	Benzo[*c*]phenanthrene	195-19-7				10				Fertuck et al. (2001)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (μM)**	SD of IC_{50}**	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
hERβ	Benzo[*c*]phenanthrene	195-19-7				10				Fertuck et al. (2001)
MCF-7 cells	Benzo[*a*]pyrene	50-32-8						14	1.146	Arcaro et al. (1999)
MCF-7 cells	Benzo[*e*]pyrene	192-97-2						57	1.756	Arcaro et al. (1999)
RUC	Benzyl alcohol	100-51-6	99.7			10000				Blair et al. (2000)
RUC	4-Benzyloxyphenol	103-16-2	99	250	50			0.00036	-3.440	Blair et al. (2000)
RUC	Benzylparaben	94-18-8	99	31.5	3.5			0.003	-2.540	Blair et al. (2000)
hERα	Biochanin A	491-80-5				10				Kuiper et al. (1998) [method a]
hERβ	Biochanin A	491-80-5				10				Kuiper et al. (1998) [method a]
RUC	Biochanin A	491-80-5						0.0043	-2.370	Fang et al. (2001)
RUC	Bis(*m* -acetoxy)-1,1,2-triphenylbut-1-ene	100808-56-8						12	1.079	Jordan et al. (1986)
RUC	Bis(*p* -acetoxy)-1,1,2-triphenylbut-1-ene	100808-54-6						73	1.863	Jordan et al. (1986)
RUC	Bisdesoxyestradiol	1217-09-0		5				0.1	-1.000	Elsby et al. (2000)
RUC	1,1-Bis-(4-hydroxyphenyl) ethane	2081-08-5	97					0.0009	-3.046	Perez et al. (1998)
RUC	4,4-Bis(4-hydroxyphenyl) heptane	7425-79-8	97					0.15	-0.824	Perez et al. (1998)
MCF-7 cells	3,4-Bis(3-hydroxyphenyl)- hexane	68266-24-0						20	1.301	Stoessel and Leclerq (1986)
MCF-7 cytosol	3,4-Bis(3-hydroxyphenyl)- hexane	68266-24-0						10	1.000	Stoessel and Leclerq (1986)
RUC	3,3-Bis(4-hydroxyphenyl) pentane	3600-64-4	97					0.18	-0.745	Perez et al. (1998)
RUC	1,1-Bis(4-hydroxyphenyl) propane	1576-13-2	97					0.15	-0.824	Perez et al. (1998)
RUC	2,2-Bis(4-hydroxyphenyl) propanol	142648-65-5	97					0.0075	-2.125	Perez et al. (1998)
GST-aERdef	(2,2-Bis(*p* -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0		0.064	0.017			4.8	0.681	Matthews et al. (2000)
GST-cERdef	(2,2-Bis(*p* -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0		0.068	0.022			4.8	0.681	Matthews et al. (2000)
GST-mERαdef	(2,2-Bis(*p* -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0		0.22	0.02			1.2	0.079	Matthews et al. (2000)
GST-rtERdef	(2,2-Bis(*p* -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0		0.024	0.001			14	1.146	Matthews et al. (2000)
hERα-FP	(2,2-Bis(*p* -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0	99	0.75				1.7	0.230	Bolger et al. (1998)
GST-hERαdef	(2,2-Bis(*p* -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0		0.25	0.08			1.2	0.079	Matthews et al. (2000)
hERα	2,2-Bis(*p* -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0	> 97	*1*				0.4	-0.398	Gaido et al. (1999)
hERβ	2,2-Bis(*p* -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0	> 97					2	0.301	Gaido et al. (1999)
MUC	2,2-Bis(*p* -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0	> 99	*0.15*				*1.2*	*0.079*	Shelby et al. (1996)
RUC	2,2-Bis(*p* -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0					0.05	5.2	0.716	Waller et al. (1996)
RUC	2,2-Bis(*p* -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0	98	0.355	0.015			0.25	-0.600	Blair et al. (2000)
RUC	2,2-Bis(*p* -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0		0.141			0.053	0.75	-0.122	Laws et al. (2000)
GST-aERdef	Bisphenol A	80-05-7		2.4	1.6			0.13	-0.886	Matthews et al. (2000)
GST-cERdef	Bisphenol A	80-05-7		7.3	1.9			0.044	-1.357	Matthews et al. (2000)
GST-hERαdef	Bisphenol A	80-05-7		36	16			0.008	-2.097	Matthews et al. (2000)
GST-mERαdef	Bisphenol A	80-05-7		31	7			0.0086	-2.066	Matthews et al. (2000)
GST-rtERdef	Bisphenol A	80-05-7		1.6	0.3			0.21	-0.678	Matthews et al. (2000)
hERα	Bisphenol A	80-05-7					0.20	0.05	-1.300	Kuiper et al. (1997)
hERα	Bisphenol A	80-05-7						0.01	-2.000	Kuiper et al. (1998) [method a]
hERα	Bisphenol A	80-05-7		150				0.003	-2.48	Morito et al. (2001)
hERα-FP	Bisphenol A	80-05-7	99	32				0.04	-1.398	Bolger et al. (1998)
hERα-FP	Bisphenol A	80-05-7	> 99	100				0.01	-2.000	Hashimoto et al. (2000)
hERβ	Bisphenol A	80-05-7						0.01	-2.000	Kuiper et al. (1998) [method a]
hERβ	Bisphenol A	80-05-7		8				0.063	-1.20	Morito et al. (2001)
MCF-7 cells	Bisphenol A	80-05-7	100					0.006	-2.222	Nagel et al. (1997)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (µM)**	SD of IC_{50}**	HDT (µM)	Ki (µM)**	RBA***	log RBA***	Reference
MCF-7 cytosol	Bisphenol A	80-05-7		*0.9*				1	0.000	Dodge et al. (1996)
MUC	Bisphenol A	80-05-7	99.9	26	11			0.01	-1.939	Matthews et al. (2001)
RBC	Bisphenol A	80-05-7	99	1.6				0.0013	-2.886	Andersen et al. (1999)
rERβ	Bisphenol A	80-05-7					0.035	0.33	-0.481	Kuiper et al. (1997)
RUC	Bisphenol A	80-05-7	99	4.192			1.57	0.026	-1.590	Laws et al. (2000)
RUC	Bisphenol A	80-05-7		*10*				0.12	-0.921	Olea et al. (1996)
RUC	Bisphenol A	80-05-7					1.45	0.1793	-0.746	Waller et al. (1996)
RUC	Bisphenol A	80-05-7	99	11.7	6.4			0.008	-2.110	Blair et al. (2000)
RUC	Bisphenol A	80-05-7						0.056	-1.252	Perez et al. (1998)
RUC	Bisphenol A bis(chloroformate)	2024-88-6						0.023	-1.638	Perez et al. (1998)
RUC	Bisphenol A diglycidyl ether	1675-54-3				100				Olea et al. (1996)
RUC	Bisphenol A diglycidyl ether	1675-54-3				*200*				Perez et al. (1998)
hERα-FP	Bisphenol A diglycidyl ether dimethacrylate	1565-94-2	99			5000				Hashimoto et al. (2000)
RUC	Bisphenol A diglycidyl ether dimethacrylate	1565-94-2				100				Olea et al. (1996)
RUC	Bisphenol A diglycidyl ether dimethacrylate	1565-94-2				*200*				Perez et al. (1998)
RBC	Bisphenol A dimethacrylate	3253-39-2	99.7	4.3				0.00047	-3.328	Andersen et al. (1999)
RUC	Bisphenol A dimethacrylate	3253-39-2		*300*				0.033	-1.481	Olea et al. (1996)
RUC	Bisphenol A dimethacrylate	3253-39-2						0.0015	-2.824	Perez et al. (1998)
RUC	Bisphenol A ethoxylate	68140-85-2				*200*				Perez et al. (1998)
RUC	Bisphenol A ethoxylate diacrylate	64401-02-1						0.0005	-3.301	Perez et al. (1998)
MUC	Bisphenol A glucuronide					100				Matthews et al. (2001)
RUC	Bisphenol A propoxylate	37353-75-6				*200*				Perez et al. (1998)
RUC	Bisphenol AF	1478-61-1	97					1	0.000	Perez et al. (1998)
RUC	Bisphenol B	77-40-7		1.05	0.46			0.086	-1.070	Blair et al. (2000)
RUC	Bisphenol B	77-40-7	97					0.15	-0.824	Perez et al. (1998)
RUC	Bisphenol C	79-97-0	97					0.25	-0.602	Perez et al. (1998)
MCF-7 cells	Bisphenol C 2	14868-03-2						0.3	-0.523	Stoessel and Leclerq (1986)
MCF-7 cytosol	Bisphenol C 2	14868-03-2						2	0.301	Stoessel and Leclerq (1986)
RUC	Bisphenol C 2	14868-03-2	98	0.034	0.004			2.64	0.420	Blair et al. (2000)
RUC	Bisphenol E	6052-84-2		2.45	0.35			0.037	-1.440	Blair et al. (2000)
RUC	2,2'-Bisphenol F	2467-02-9	98			10				Blair et al. (2000)
RUC	4,4'-Bisphenol F	620-92-8	98	95	5			0.0009	-3.020	Blair et al. (2000)
RUC	4,4'-Bisphenol F	620-92-8						0.15	-0.824	Perez et al. (1998)
RUC	Bisphenol S	80-09-1	99	105	35			0.0009	-3.070	Blair et al. (2000)
hERα	16α-Bromo-17β-estradiol	54982-79-5						76	1.881	Kuiper et al. (1998) [method a]
hERα	16α-Bromo-17β-estradiol	54982-79-5		0.00172				54.0	1.730	Kuiper et al. (1998) [method b]
hERβ	16α-Bromo-17β-estradiol	54982-79-5						10	1.000	Kuiper et al. (1998) [method a]
hERβ	16α-Bromo-17β-estradiol	54982-79-5		0.0063				16.8	1.230	Kuiper et al. (1998) [method b]
MCF-7 cells	1,3-Butanediol, 4-[4-(1,2,3,4-tetrahydro-6-hydroxy-2-phenyl-1-naphthalenyl)phenoxy]-	107144-85-4						7	0.845	Stoessel and Leclerq (1986)
MCF-7 cytosol	1,3-Butanediol, 4-[4-(1,2,3,4-tetrahydro-6-hydroxy-2-phenyl-1-naphthalenyl) phenoxy]-	107144-85-4						30	1.477	Stoessel and Leclerq (1986)
MCF-7 cells	1,3-Butanediol, 4-[4-[1,2,3,4-tetrahydro-6-methoxy-2-phenyl-1-naphthenyl)-phenoxy] -	107163-56-4						0.06	-1.222	Stoessel and Leclerq (1986)
MCF-7 cytosol	1,3-Butanediol, 4-[4-[1,2,3,4-tetrahydro-6-methoxy-2-phenyl-1-naphthenyl)-phenoxy] -	107163-56-4						0.1	-1.000	Stoessel and Leclerq (1986)
RUC	Butolame	150748-23-5		*6*				*0.14*	-0.854	Jaimez et al. (2000)
RUC	Butyl 4-aminobenzoate	94-25-7	99			100				Blair et al. (2000)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (µM)**	SD of IC_{50}**	HDT (µM)	Ki (µM)**	RBA***	log RBA***	Reference
RUC	*n* -Butylbenzene	104-51-8	99			200				Blair et al. (2000)
RUC	*sec* -Butylbenzene	135-98-8	99			1000				Blair et al. (2000)
GST-aERdef	Butyl benzyl phthalate	85-68-7				*100*				Matthews et al. (2000)
GST-cERdef	Butyl benzyl phthalate	85-68-7				*100*				Matthews et al. (2000)
GST-hERαdef	Butyl benzyl phthalate	85-68-7				*100*				Matthews et al. (2000)
GST-mERαdef	Butyl benzyl phthalate	85-68-7				*100*				Matthews et al. (2000)
GST-rtERdef	Butyl benzyl phthalate	85-68-7				100				Matthews et al. (2000)
hERα-FP	Butyl benzyl phthalate	85-68-7	98	*105*				*0.012*	-1.921	Bolger et al. (1998)
hERα-FP	Butyl benzyl phthalate	85-68-7	97	5000				0.0002	-3.699	Hashimoto et al. (2000)
RBC	Butyl benzyl phthalate	85-68-7	97			100				Andersen et al. (1999)
RUC	Butyl benzyl phthalate	85-68-7	98			1000				Blair et al. (2000)
RUC	Butyl benzyl phthalate	85-68-7				*500*				Elsby et al. (2000)
RUC	Butyl benzyl phthalate	85-68-7				100				Fang et al. (2001)
RUC	Butyl benzyl phthalate	85-68-7					76.38	0.0034	-2.470	Waller et al. (1996)
RUC	Butyl benzyl phthalate	85-68-7	98.5	36				0.000036	-4.444	Zacharewski et al. (1998)
RUC	Butylparaben	94-26-8	99	105	35			0.0009	-3 07	Blair et al. (2000)
RUC	Butylparaben	94-26-8	> 99	10				0.002	-2.699	Routledge et al. (1998)
RUC	2-*sec* -Butylphenol	89-72-5	98	315	5			0.00029	-3.540	Blair et al. (2000)
RUC	2-*tert* -Butylphenol	88-18-6					232	0.0011	-2.959	Waller et al. (1996)
RUC	3-*tert* -Butylphenol	585-34-2					395	0.0007	-3.155	Waller et al. (1996)
RUC	4-*sec* -Butylphenol	99-71-8	96	210	30			0.00043	-3 37	Blair et al. (2000)
hERα	4-*tert* -Butylphenol	98-54-4				10				Kuiper et al. (1998) [method a]
hERβ	4-*tert* -Butylphenol	98-54-4				10				Kuiper et al. (1998) [method a]
RUC	4-*tert* -Butylphenol	98-54-4					161	0.0016	-2.796	Waller et al. (1996)
RUC	4-*tert* -Butylphenol	98-54-4	99	368	83			0.00024	-3.610	Blair et al. (2000)
hERα-FP	Butyl phthalyl *n* -butyl glycolate	85-70-1	93			5000				Hashimoto et al. (2000)
RUC	Caffeine	58-08-2	100			100				Blair et al. (2000)
RUC	Carbaryl	63-25-2	99			100				Blair et al. (2000)
RUC	Carbofuran	1563-66-2	98			100				Blair et al. (2000)
RUC	Castor oil	8001-79-4				100				Blair et al. (2000)
RUC	(±)-Catechin	7295-85-4				100				Fang et al. (2001)
RUC	Chalcone	94-41-7						0.0015	-2.820	Fang et al. (2001)
hERα	Chlordane	57-74-9	95 - 99			10				Arcaro et al. (2000)
RUC	α-Chlordane	5103-71-9				1000				Blair et al. (2000)
RBC	Chlormequat chloride	999-81-5	97			100				Andersen et al. (1999)
hERα-FP	2-Chloro-4-amino-6-isopropylamino-1,3,5-triazine	6190-65-4	99.7	951	105			0.00002	-4.699	Hanioka et al. (1999)
MUC	2'-Chloro-4,4'-biphenyldiol	56858-70-9	> 98	0.0900				1.11	0.045	Korach et al. (1988)
MUC	2-Chloro-4-biphenylol	23719-22-4	> 98	2.50				0.040	-1.398	Korach et al. (1988)
RUC	2-Chloro-4-biphenylol	23719-22-4	95	52.5	25.5			0.002	-2.770	Blair et al. (2000)
MUC	4-Chloro-4'-biphenylol	28034-99-3	> 98	3.9				0.026	-1.585	Korach et al. (1988)
MUC	4-Chloro-4'-biphenylol	28034-99-3					5.57	0.047	-1.330	Waller et al. (1996)
RUC	4-Chloro-4'-biphenylol	28034-99-3	95	13.5	1.5			0.007	-2.180	Blair et al. (2000)
RUC	4-Chloro-*m* -cresol	59-50-7	99	215	15			0.00042	-3.380	Blair et al. (2000)
RUC	2-Chloro-4,6-diamino-*S* -triazine	3397-62-4					1000	0.0003	-3.523	Waller et al. (1996)
hERα-FP	2-Chloro-4-ethylamino-6-amino-1,3,5-triazine	1007-28-9	99.2			2000				Hanioka et al. (1999)
hERα-FP	2-Chloro-4-ethylamino-6-(1-hydroxyisopropyl)amino-1,3,5-triazine	142179-80-4	99.5			2000				Hanioka et al. (1999)
hERα-FP	2-Chloro-4-isopropylamino-6-(1-hydroxyisopropylamino)-1,3,5-triazine	142200-36-0	99.1			2000				Hanioka et al. (1999)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (μM)**	SD of IC_{50}**	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
MCF-7 cells	11β-Chloromethylestradiol	71794-60-0						96	1.982	Stoessel and Leclerq (1986)
MCF-7 cytosol	11β-Chloromethylestradiol	71794-60-0						100	2.000	Stoessel and Leclerq (1986)
RUC	2-Chloro-4-methylphenol	6640-27-3	97	415	175			0.00022	-3.660	Blair et al. (2000)
RUC	4-Chloro-2-methylphenol	1570-64-5	97	425	105			0.00021	-3.670	Blair et al. (2000)
RUC	2-Chlorophenol	95-57-8	99			200				Blair et al. (2000)
RUC	4-Chlorophenol	106-48-9	90	25.5	1.5			0.004	-2.450	Blair et al. (2000)
RUC	Chlorotamoxifen	77588-46-6				10				Allen et al. (1980)
RUC	Cholesterol	57-88-5	99			1000				Blair et al. (2000)
GST-hERαdef	Chrysene	218-01-9				10				Fertuck et al. (2001)
hERβ	Chrysene	218-01-9				10				Fertuck et al. (2001)
MCF-7 cells	Chrysene	218-01-9						13	1.114	Arcaro et al. (1999)
RUC	Chrysene	218-01-9	98			10				Blair et al. (2000)
hERα	Chrysin	480-40-0				10				Kuiper et al. (1998) [method a]
hERβ	Chrysin	480-40-0				10				Kuiper et al. (1998) [method a]
RUC	Chrysin	480-40-0				100				Fang et al. (2001)
RUC	Cineole	470-82-6	90			10000				Blair et al. (2000)
RUC	Cinnamic acid	621-82-9	99.9			1000				Blair et al. (2000)
RBC	*cis* -Clomiphene	15690-55-8						0.12	-0.910	Korenman (1970)
hERα	*trans* -Clomiphene	911-45-5					0.0009	25	1.398	Kuiper et al. (1997)
RBC	*trans* -Clomiphene	911-45-5						0.0081	-2.092	Korenman (1970)
rERβ	*trans* -Clomiphene	911-45-5					0.0012	12	1.079	Kuiper et al. (1997)
RUC	Clomiphene citrate	50-41-9	98	0.125	0.075			0.72	-0.140	Blair et al. (2000)
RBC	Colchicine	64-86-8	95			100				Andersen et al. (1999)
hERα	Corticosterone	50-22-6				100				Kuiper et al. (1997)
rERβ	Corticosterone	50-22-6				100				Kuiper et al. (1997)
RUC	Corticosterone	50-22-6	95			100				Blair et al. (2000)
MUC	Cortisol	50-23-7				10				Korach (1979)
GST-aERdef	Coumestrol	479-13-0		0.1	0.04			3.1	0.491	Matthews et al. (2000)
GST-cERdef	Coumestrol	479-13-0		0.46	0.1			0.7	-0.155	Matthews et al. (2000)
GST-hERαdef	Coumestrol	479-13-0		0.036	0.03			0.81	-0.092	Matthews et al. (2000)
GST-mERαdef	Coumestrol	479-13-0		0.8	0.32			0.33	-0.481	Matthews et al. (2000)
GST-rtERdef	Coumestrol	479-13-0		1.4	0.1			0.24	-0.620	Matthews et al. (2000)
hERα	Coumestrol	479-13-0					0.00014	94	1.970	Kuiper et al. (1997)
hERα	Coumestrol	479-13-0						20	1.301	Kuiper et al. (1998) [method a]
hERα	Coumestrol	479-13-0		0.0027				34	1.532	Kuiper et al. (1998) [method b]
hERα-FP	Coumestrol	479-13-0		0.109	0.001			12	1.079	Nikov et al. (2000)
hERβ	Coumestrol	479-13-0						140	2.146	Kuiper et al. (1998) [method a]
hERβ	Coumestrol	479-13-0		0.0011				100	2.000	Kuiper et al. (1998) [method b]
MCF-7 cytosol	Coumestrol	479-13-0		*0.01*				13	1.114	Dodge et al. (1996)
rERβ	Coumestrol	479-13-0					0.00007	185	2.267	Kuiper et al. (1997)
RUC	Coumestrol	479-13-0						0.9	-0.045	Fang et al. (2001)
RUC	Coumestrol	479-13-0					0.093	2.82	0.450	Waller et al. (1996)
RUC	*p* -Cumyl phenol	599-64-4						0.005	-2.301	Fang et al. (2001)
MCF-7 cells	Cyclofenil diphenol	5189-40-2						0.5	-0.301	Stoessel and Leclerq (1986)
MCF-7 cytosol	Cyclofenil diphenol	5189-40-2						5	0.699	Stoessel and Leclerq (1986)
MCF-7 cells	Cycloprop[14*R* ,15α]estra-1,3,5(10)-triene 3,17β-diol, 3',15-dihydro-	73860-54-5						39	1.591	Stoessel and Leclerq (1986)
MCF-7 cytosol	Cycloprop[14*R* ,15α]estra-1,3,5(10)-triene 3,17β-diol, 3',15-dihydro-	73860-54-5						45	1.653	Stoessel and Leclerq (1986)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (µM)**	SD of IC_{50}**	HDT (µM)	Ki (µM)**	RBA***	log RBA***	Reference
MCF-7 cells	Cycloprop[14*S* ,15b]estra-1,3,5(10)-triene-3,17β-diol, 3', 15-dihydro-	105455-76-3						81	1.908	Stoessel and Leclerq (1986)
MCF-7 cytosol	Cycloprop[14*S* ,15b]estra-1,3,5(10)-triene-3,17β-diol, 3', 15-dihydro-	105455-76-3						100	2.000	Stoessel and Leclerq (1986)
hERα-FP	Cypermethrin	52315-07-8	> 93			10				Saito et al. (2000)
hERα	Daidzein	486-66-8						0.1	-1.000	Kuiper et al. (1998) [method a]
hERα	Daidzein	486-66-8		0.42				0.2	-0.699	Kuiper et al. (1998) [method b]
hERα	Daidzein	486-66-8		23				0.022	-1.66	Morito et al. (2001)
hERα-FP	Daidzein	486-66-8		7	1			0.2	-0.699	Nikov et al. (2000)
hERβ	Daidzein	486-66-8						0.5	-0.301	Kuiper et al. (1998) [method a]
hERβ	Daidzein	486-66-8		0.1				1	0.000	Kuiper et al. (1998) [method b]
hERβ	Daidzein	486-66-8		0.45				1.11	0.05	Morito et al. (2001)
RUC	Daidzein	486-66-8						0.023	-1.638	Fang et al. (2001)
RUC	*m,p* '-DDD	4329-12-8				*100*				Nelson (1974)
hERα	*o,p* '-DDD	53-19-0				50				Klotz et al. (1996)
hERα	*o,p* '-DDD	53-19-0				10				Kuiper et al. (1998) [method a]
hERβ	*o,p* '-DDD	53-19-0				10				Kuiper et al. (1998) [method a]
RUC	*o,p* '-DDD	53-19-0	99.2			300				Blair et al. (2000)
RUC	*o,p* '-DDD	53-19-0		*10*				*0.009*	-2.045	Nelson (1974)
hERα	*p,p* '-DDD	72-54-8		11				0.009	-2.050	Klotz et al. (1996)
hERα	*p,p* '-DDD	72-54-8				10				Kuiper et al. (1998) [method a]
hERβ	*p,p* '-DDD	72-54-8				10				Kuiper et al. (1998) [method a]
RUC	*p,p* '-DDD	72-54-8					1000	0.0003	-3.523	Waller et al. (1996)
RUC	*p,p* '-DDD	72-54-8	98.5			100				Blair et al. (2000)
RUC	*p,p* '-DDD	72-54-8				*100*				Nelson (1974)
hERα	*o,p* '-DDE	3424-82-6				10				Kuiper et al. (1998) [method a]
hERβ	*o,p* '-DDE	3424-82-6				10				Kuiper et al. (1998) [method a]
GST-aERdef	*o,p* '-DDE	3424-82-6				*100*				Matthews et al. (2000)
GST-cERdef	*o,p* '-DDE	3424-82-6				*100*				Matthews et al. (2000)
GST-hERαdef	*o,p* '-DDE	3424-82-6				*100*				Matthews et al. (2000)
GST-mERαdef	*o,p* '-DDE	3424-82-6				*100*				Matthews et al. (2000)
GST-rtERdef	*o,p* '-DDE	3424-82-6		3.2	1			0.11	-0.959	Matthews et al. (2000)
RUC	*o,p'* -DDE	3242-82-6	99.8			500				Blair et al. (2000)
RUC	*o,p* '-DDE	3242-82-6		*100*				*0.0009*	-3.045	Nelson (1974)
hERα	*p,p* '-DDE	72-55-9				10				Kuiper et al. (1998) [method a]
hERβ	*p,p* '-DDE	72-55-9				10				Kuiper et al. (1998) [method a]
RUC	*p,p* ' DDE	72-55-9				*100*				Nelson (1974)
RUC	*p,p* ' DDE	72-55-9					1000	0.0003	-3.523	Waller et al. (1996)
GST-aERdef	*p,p* '-DDE	72-55-9				*100*				Matthews et al. (2000)
GST-cERdef	*p,p* '-DDE	72-55-9				100				Matthews et al. (2000)
GST-hERαdef	*p,p* '-DDE	72-55-9				*100*				Matthews et al. (2000)
GST-mERαdef	*p,p* '-DDE	72-55-9				*100*				Matthews et al. (2000)
GST-rtERdef	*p,p* '-DDE	72-55-9		8	0.6			0.042	-1.377	Matthews et al. (2000)
RBC	*p,p* '-DDE	72-55-9	99.7			100				Andersen et al. (1999)
RUC	*p,p* '-DDE	72-55-9	99.4			100				Blair et al. (2000)
GST-rtERdef	*o,p* '-DDT	789-02-6		0.78	0.01			0.43	-0.367	Matthews et al. (2000)
hERα	*o,p* '-DDT	789-02-6		1				0.1	-1.000	Klotz et al. (1996)
hERα	*o,p* '-DDT	789-02-6						0.01	-2.000	Kuiper et al. (1998) [method a]
hERβ	*o,p* '-DDT	789-02-6						0.02	-1.699	Kuiper et al. (1998) [method a]
MUC	*o,p* '-DDT	789-02-6	> 99	*0.875*				*0.210*	*-0.678*	Shelby et al. (1996)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (µM)**	SD of IC_{50}**	HDT (µM)	Ki (µM)**	RBA***	log RBA***	Reference
RBC	*o,p* '-DDT	789-02-6	99.8	3.4				0.00059	-3.229	Andersen et al. (1999)
RUC	*o,p* '-DDT	789-02-6		13.1			0.44	0.011	-1.959	McBlain (1987)
RUC	*o,p* '-DDT	789-02-6					2.89	0.09	-1.046	Waller et al. (1996)
hERα-FP	*o,p* '-DDT	789-02-6	99	2.7				0.4	-0.398	Bolger et al. (1998)
GST-aERdef	*o,p* '-DDT	789-02-6				*100*				Matthews et al. (2000)
GST-cERdef	*o,p* '-DDT	789-02-6		3.7	1.2			0.086	-1.066	Matthews et al. (2000)
GST-hERαdef	*o,p* '-DDT	789-02-6				*100*				Matthews et al. (2000)
GST-mERαdef	*o,p* '-DDT	789-02-6		36	35			0.0073	-2.137	Matthews et al. (2000)
MCF-7 cytosol	*o,p* '-DDT	789-02-6		485	42			0.00031	-3.509	Soto et al. (1995)
MCF-7 cells	(-)-*o,p* '-DDT	58633-26-4		*3*				*0.013*	-1.88	Lascombe et al. (2000)
RUC	*o,p* '-DDT	789-02-6	98.5	64.3	8.9			0.001	-2.850	Blair et al. (2000)
RUC	*o,p* '-DDT	789-02-6		8.544			3.2	0.013	-1.900	Laws et al. (2000)
RUC	*o,p* '-DDT	789-02-6		*2*				*0.045*	-1.346	Nelson (1974)
RUC	(-)-*o,p* '-DDT	58633-26-4		5			0.17	0.029	-1.538	McBlain (1987)
MCF-7 cells	(+)-*o,p* '-DDT	58633-27-5		*400*				*0.0001*	-4.00	Lascombe et al. (2000)
RUC	(+)-*o,p* '-DDT	58633-27-5				20				McBlain (1987)
hERα	*p,p* '-DDT	50-29-3				10				Kuiper et al. (1998) [method a]
hERα-FP	*p,p* '-DDT	50-29-3	98	*32*				*0.041*	-1.387	Bolger et al. (1998)
hERβ	*p,p* '-DDT	50-29-3				10				Kuiper et al. (1998) [method a]
GST-aERdef	*p,p* '-DDT	50-29-3				*100*				Matthews et al. (2000)
GST-cERdef	*p,p* '-DDT	50-29-3				*100*				Matthews et al. (2000)
GST-hERαdef	*p,p* '-DDT	50-29-3				*100*			n.a	Matthews et al. (2000)
GST-mERαdef	*p,p* '-DDT	50-29-3				*100*				Matthews et al. (2000)
GST-rtERdef	*p,p* '-DDT	50-29-3		2	0.4			0.165	-0.783	Matthews et al. (2000)
RUC	*p,p* '-DDT	50-29-3					1000	0.0003	-3.523	Waller et al. (1996)
RUC	*p,p* '-DDT	50-29-3	99.2			1000				Blair et al. (2000)
RUC	*p,p* '-DDT	50-29-3				*100*				Nelson (1974)
GST-aERdef	Dehydroepiandrosterone	53-43-0				*100*				Matthews et al. (2000)
GST-cERdef	Dehydroepiandrosterone	53-43-0				*100*				Matthews et al. (2000)
GST-hERαdef	Dehydroepiandrosterone	53-43-0				*100*				Matthews et al. (2000)
GST-mERαdef	Dehydroepiandrosterone	53-43-0				*100*				Matthews et al. (2000)
GST-rtERdef	Dehydroepiandrosterone	53-43-0		12	2			0.028	-1.553	Matthews et al. (2000)
hERα	Dehydroepiandrosterone	53-43-0					0.25	0.04	-1.398	Kuiper et al. (1997)
rERβ	Dehydroepiandrosterone	53-43-0					0.16	0.07	-1.155	Kuiper et al. (1997)
RUC	14-Dehydroestradiol-17β	58699-19-7						107	2.029	Gabbard and Segaloff (1983)
MCF-7 cytosol	9, 11-Dehydroestradiol							196	2.292	Palomino et al. (1994)
RUC	14 Dehydroestradiol-17β 3-methyl ether	35664-58-7						0.8	-0.097	Gabbard and Segaloff (1983)
RUC	14-Dehydroestrone	2119-18-8						9	0.954	Gabbard and Segaloff (1983)
RUC	14-Dehydroestrone 3-methyl ether	17550-11-7				1				Gabbard and Segaloff (1983)
MCF-7 cells	3-Deoxyestradiol	2529-64-8						0.6	-0.222	Brooks et al. (1987)
MCF-7 cytosol	3-Deoxyestradiol	2529-64-8						8	0.903	Brooks et al. (1987)
RUC	3-Deoxyestradiol	2529-64-8		0.18	0.02			0.50	-0.300	Blair et al. (2000)
RUC	3-Deoxyestrone	53-45-2		14.3	5.8			0.006	-2.200	Blair et al. (2000)
MUC	(*R*)-4'-Deoxyindenestrol A	138515-00-1	> 98	0.556				0.20	-0.699	Chae et al. (1991)
MUC	(*rac*)-4'-Deoxyindenestrol A		> 98	0.0756				1.30	0.114	Chae et al. (1991)
MUC	(*S*)-4'-Deoxyindenestrol A	138514-99-5	> 98	0.0644				1.80	0.255	Chae et al. (1991)
MUC	(*R*)-5-Deoxyindenestrol A	138515-02-3	> 98	0.117				0.90	-0.046	Chae et al. (1991)
MUC	(*rac*)-5-Deoxyindenestrol A	138472-84-1	> 98	0.027				3.70	0.568	Chae et al. (1991)
MUC	(*S*)-5-Deoxyindenestrol A	138515-01-2	> 98	0.0177				5.60	0.748	Chae et al. (1991)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (μM)**	SD of IC_{50}**	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
MCF-7 cells	17-Desoxyestradiol	53-63-4						0.5	-0.301	Brooks et al. (1987)
MCF-7 cytosol	17-Desoxyestradiol	53-63-4						40	1.602	Brooks et al. (1987)
RUC	17-Desoxyestradiol	53-63-4		0.00885	0.0032			10.16	1.010	Blair et al. (2000)
RUC	17-Desoxyestradiol	53-63-4		0.00495	0.00085			18.16	1.260	Blair et al. (2000)
RUC	17-Desoxyestradiol	53-63-4		0.009				55.5	1.744	Elsby et al. (2000)
RUC	Dexamethasone	50-02-2				100				Blair et al. (2000)
RUC	1,3-Diacetoxy-17α-ethinyl-7α-methyl-1,3,5(10)-estratrien-17β-ol			*0.008*				20	1.301	Leibl and Spona (1982)
RUC	4,4'-Diaminostilbene dihydrochloride	66635-40-3	95			100				Blair et al. (2000)
MCF-7 cells	Dibenz[*ah*]anthracene	53-70-3				5				Arcaro et al. (1999)
RUC	Dibenzo-18-crown-6	14187-32-7	98			10				Blair et al. (2000)
RUC	1,3-Dibenzoyloxy-17α-ethinyl-7α-methyl-1,3,5(10)-estratrien-17β-ol			*0.022*				7.3	0.863	Leibl and Spona (1982)
RUC	1,3-Dibenzyltetramethyldisiloxane					100				Fang et al. (2001)
GST-aERdef	Dibutyl benzyl phthalate					*100*				Matthews et al. (2000)
GST-cERdef	Dibutyl benzyl phthalate					*100*				Matthews et al. (2000)
GST-hERαdef	Dibutyl benzyl phthalate					*100*				Matthews et al. (2000)
GST-mERαdef	Dibutyl benzyl phthalate					*100*				Matthews et al. (2000)
GST-rtERdef	Dibutyl benzyl phthalate			1.7	2.3			0.2	-0.699	Matthews et al. (2000)
RUC	2,6-Di-*tert* -butylphenol	128-39-2	98			100				Blair et al. (2000)
hERα-FP	Dibutyl phthalate	84-74-2	> 98			5000				Hashimoto et al. (2000)
RBC	Dibutyl phthalate	84-74-2	98			100				Andersen et al. (1999)
RUC	Dibutyl phthalate	84-74-2	99			1000				Blair et al. (2000)
RUC	Dibutyl phthalate	84-74-2					100.46	0.0026	-2.590	Waller et al. (1996)
RUC	Dibutyl phthalate	84-74-2	99.9	47				0.0028	-2.553	Zacharewski et al. (1998)
RUC	2,4'-Dichlorobiphenyl	34883-43-7	99	365	115			0.0002	-3.610	Blair et al. (2000)
hERα	2,5-Dichlorobiphenyl	34883-39-1	≥ 99%			50				Vakharia and Gierthy (2000)
MUC	2',6'-Dichloro-4-biphenylol	79881-33-7	> 98	0.3880				0.26	-0.588	Korach et al. (1988)
hERα	3,4-Dichlorobiphenyl	2974-92-7	≥ 99%			50				Vakharia and Gierthy (2000)
hERα	3,5-Dichlorobiphenyl	34883-41-5	≥ 99%			50				Vakharia and Gierthy (2000)
RUC	4,4'-Dichlorobiphenyl	2050-68-2	98.6			300				Blair et al. (2000)
hERα	2,5-Dichloro-2'-biphenylol	53905-30-9	≥ 99%			50				Vakharia and Gierthy (2000)
hERα	2,5-Dichloro-3'-biphenylol	53905-29-6	≥ 99%	50				0.002	-2.700	Vakharia and Gierthy (2000)
hERα	2',5'-Dichloro-4-biphenylol	53905-28-5	≥ 99%	3				0.033	-1.480	Vakharia and Gierthy (2000)
MUC	2',5'-Dichloro-4-biphenylol	53905-28-5	> 98	0.5060				0.198	-0.703	Korach et al. (1988)
MUC	2',5'-Dichloro-4-biphenylol	53905-28-5					0.36	0.72	-0.140	Waller et al. (1996)
RUC	2',5'-Dichloro-4-biphenylol	53905-28-5	95	2.5	0.3			0.036	-1.440	Blair et al. (2000)
hERα	3,4-Dichloro-2'-biphenylol	209613-97-8	95 - 99			50				Vakharia and Gierthy (2000)
hERα	3,4-dichloro-3'-biphenylol	14962-34-6	95 - 99			50				Vakharia and Gierthy (2000)
hERα	3,4-dichloro-4'-biphenylol	53890-77-0	95 - 99	0.33				0.30	-0.519	Vakharia and Gierthy (2000)
hERα	3,5-Dichloro-2'-biphenylol		≥ 99%			50				Vakharia and Gierthy (2000)
hERα	3,5-Dichloro-4'-biphenylol		≥ 99%			50				Vakharia and Gierthy (2000)
RUC	3,5-Dichloro 2-hydroxy-2-methylbut-3-enanalide	16776-82-1	> 99			*200*				Laws et al. (1996)
RUC	3,5-Dichloro 2-hydroxy-2-methylbut-3-enanalide	16776-82-1					1000	0.0003	-3.523	Waller et al. (1996)
RUC	2,4-Dichlorophenoxyacetic acid	94-75-7	99			100				Blair et al. (2000)
RUC	2-[[(3,5-Dichlorophenyl)amino]-carbamoyl]oxy]-2-methyl-3-butenoic acid	119209-27-7					1000	0.0003	-3.523	Waller et al. (1996)
RUC	2-[[(3,5-Dichlorophenyl)amino]-carbamoyl]oxy]-2-methyl-3-butenoic acid	119209-27-7				*500*				Laws et al. (1996)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (µM)**	SD of IC_{50}**	HDT (µM)	Ki (µM)**	RBA***	log RBA***	Reference
hERα	Dieldrin	60-57-1	95 - 99			10				Arcaro et al. (2000)
hERα-FP	Dieldrin	60-57-1	98.8	*2485*				*0.0005*	-3.301	Bolger et al. (1998)
MUC	Dieldrin	60-57-1	Technical grade			10				Ramamoorthy et al. (1997a)
RUC	Dieldrin	60-57-1	98			100				Blair et al. (2000)
RUC	Dieldrin	60-57-1	90			100				Blair et al. (2000)
hERα	Dienestrol	84-17-3					0.00005	223	2.348	Kuiper et al. (1997)
rERβ	Dienestrol	84-17-3					0.00003	404	2.606	Kuiper et al. (1997)
RUC	Dienestrol	84-17-3	99	0.0024	0			37.46	1.570	Blair et al. (2000)
MUC	α-Dienestrol	13029-44-2		0.005	0.0008			32	1.500	Korach et al. (1978)
MUC	β-Dienestrol	35495-11-5		0.367	0.072			0.44	-0.357	Korach et al. (1978)
RUC	1,3-Diethyl-6,4'-dihydroxy-2-phenylindene							79	1.898	Anstead et al. (1989)
hERα-FP	Di-2-ethylhexyl adipate	103-23-1	99			5000				Hashimoto et al. (2000)
hERα-FP	Diethylhexyl phthalate	117-81-7	99			5000				Hashimoto et al. (2000)
RUC	Diethylhexyl phthalate	117-81-7	99			1000				Blair et al. (2000)
RUC	Diethylhexyl phthalate	117-81-7	99.9			1000				Zacharewski et al. (1998)
RUC	1,3-Diethyl-4-hydroxy-2-phenylindene							9.3	0.968	Anstead et al. (1989)
RUC	1,3-Diethyl-6-hydroxy 2-phenylindene							2.2	0.342	Anstead et al. (1989)
RUC	*meso-p* -(α,β-Diethyl-*p* -methylphenethyl)phenol	267408-76-4		0.0225	0.0075			4.00	0.600	Blair et al. (2000)
RUC	Diethyl phthalate	84-66-2	99			1000				Blair et al. (2000)
GST-aERdef	Diethylstilbestrol	56-53-1		0.0029	0.0001			107	2.029	Matthews et al. (2000)
GST-cERdef	Diethylstilbestrol	56-53-1		0.0025	0.0006			130	2.114	Matthews et al. (2000)
GST-hERαdef	Diethylstilbestrol	56-53-1		0.0032	0.0001			91	1.959	Matthews et al. (2000)
GST-mERαdef	Diethylstilbestrol	56-53-1		0.0032	0.0005			84	1.924	Matthews et al. (2000)
GST-rtERdef	Diethylstilbestrol	56-53-1		0.002	0.0001			165	2.217	Matthews et al. (2000)
hERα	Diethylstilbestrol	56-53-1					0.00004	468	2.670	Kuiper et al. (1997)
hERα	Diethylstilbestrol	56-53-1						236	2.373	Kuiper et al. (1998) [method a]
hERα	Diethylstilbestrol	56-53-1		0.0075				66.7	1.82	Morito et al. (2001)
hERα-FP	Diethylstilbestrol	56-53-1	99	0.011				118	2.072	Bolger et al. (1998)
hERα-FP	Diethylstilbestrol	56-53-1		0.01	0.0005			130	2.114	Nikov et al. (2001)
hERα-FP	Diethylstilbestrol	56-53-1		0.0035				160	2.204	Parker et al. (2000)
hERα-FP	Diethylstilbestrol	56-53-1	> 93	*0.07*				*57*	1.756	Saito et al. (2000)
hERβ	Diethylstilbestrol	56-53-1						221	2.344	Kuiper et al. (1998) [method a]
hERβ	Diethylstilbestrol	56-53-1		*0.005*				100	2.00	Morito et al. (2001)
MCF-7 cells	Diethylstilbestrol	56-53-1						84	1.924	Stoessel and Leclerq (1986)
MCF-7 cytosol	Diethylstilbestrol	56-53-1					0.00018	100	2.000	Rijks et al. (1996)
MCF-7 cytosol	Diethylstilbestrol	56-53-1						100	2.000	Stoessel and Leclerq (1986)
MUC	Diethylstilbestrol	56-53-1						90	1.950	Korach (1979)
MUC	Diethylstilbestrol	56-53-1		0.002	0.0003			80	1.900	Korach et al. (1978)
MUC	Diethylstilbestrol	56-53-1		0.001	0.0001			100	2.000	Korach et al. (1979)
MUC	Diethylstilbestrol	56-53-1		0.5	0.1			320	2.510	Korach et al. (1985)
MUC	Diethylstilbestrol	56-53-1		0.0004				250	2.398	Korach et al. (1988)
MUC	Diethylstilbestrol	56-53-1						286	2.460	Korach et al. (1989)
MUC	Diethylstilbestrol	56-53-1	> 99	*0.0016*				*113*	*2.050*	Shelby et al. (1996)
RBC	Diethylstilbestrol	56-53-1	99	7				2857	3.456	Andersen et al. (1999)
RBC	Diethylstilbestrol	56-53-1						246	2.391	Korenman (1969)
rERβ	Diethylstilbestrol	56-53-1					0.00004	295	2.470	Kuiper et al. (1997)
RUC	Diethylstilbestrol	56-53-1		*100*				*0.003*	-2.523	Ashby et al. (1999)
RUC	Diethylstilbestrol	56-53-1		0.000225	0.000005			399.56	2.600	Blair et al. (2000)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (µM)**	SD of IC_{50}**	HDT (µM)	Ki (µM)**	RBA***	log RBA***	Reference
RUC	Diethylstilbestrol	56-53-1		*0.0001*				*5000*	3.699	Elsby et al. (2000)
RUC	Diethylstilbestrol	56-53-1		0.0014				214	2.330	Laws et al. (1996)
RUC	Diethylstilbestrol	56-53-1		0.0005			0.0002	200	2.300	Laws et al. (2000)
RUC	Diethylstilbestrol	56-53-1		0.00019				100	2.000	Liu et al. (1994)
RUC	Diethylstilbestrol	56-53-1		0.00031			0.00001	471	2.673	McBlain (1987)
RUC	Diethylstilbestrol	56-53-1		*0.001*				90	1.950	Nelson (1974)
RUC	Diethylstilbestrol	56-53-1					0.00016	100	2.000	Rijks et al. (1996)
RUC	Diethylstilbestrol	56-53-1	> 99	0.0002				100	2.000	Routledge et al. (1998)
RUC	Diethylstilbestrol	56-53-1					0.0006998	371	2.569	Waller et al. (1996)
MCF-7 cells	3,3'-Diethylstilbestrol	5959-71-7						17.5	1.243	Stoessel and Leclerq (1986)
MCF-7 cytosol	3,3'-Diethylstilbestrol	5959-71-7						3	0.477	Stoessel and Leclerq (1986)
RUC	Diethylstilbestrol dimethyl ether	130-79-0		1.6	0.3			0.056	-1.250	Blair et al. (2000)
MUC	Diethylstilbestrol epoxide	6052-82-0		0.017	0.002			9.4	0.970	Korach et al. (1978)
MUC	Diethylstilbestrol-phenanthrene			0.6	0.173			0.27	-0.569	Korach et al. (1978)
hERα	(*rac*) 5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							14	1.146	Meyers et al. (1999)
hERβ	(*rac*) 5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							67	1.830	Meyers et al. (1999)
hERα	(5*R* ,11*R*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							23	1.361	Meyers et al. (1999)
hERβ	(5*R* ,11*R*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							144	2.160	Meyers et al. (1999)
hERβ	(5*S* ,11*S*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							14	1.150	Meyers et al. (1999)
hERα	(5*S* ,11*S*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							0.9	-0.046	Meyers et al. (1999)
hERα	(*trans*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							221	2.344	Meyers et al. (1999)
hERβ	(*trans*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							432	2.640	Meyers et al. (1999)
RUC	Dihexyl phthalate	84-75-3	99.6			1000				Zacharewski et al. (1998)
RUC	5,6-Dihydro-8-[2-(dimethylamino)ethoxy]-12-ethyl-11-phenyl-dibenzo[a,e]-cyclooctene, hydrate (1:4)	85850-78-8						0.22	-0.658	Acton et al. (1983)
hERα	Dihydrogenistein	21554-71-2		3.5				0.143	-0.84	Morito et al. (2001)
hERβ	Dihydrogenistein	21554-71-2		0.027				18.5	1.27	Morito et al. (2001)
hERβ	Dihydroglycitein	94105-88-1		22				0.023	-1.64	Morito et al. (2001)
GST-aERdef	5α-Dihydrotestosterone	521-18-6		0.82	0.12			0.38	-0.420	Matthews et al. (2000)
GST-cERdef	5α-Dihydrotestosterone	521-18-6		38	6			0.0085	-2.071	Matthews et al. (2000)
GST-hERαdef	5α-Dihydrotestosterone	521-18-6		5.9	0.9			0.049	-1.310	Matthews et al. (2000)
GST-mERαdef	5α-Dihydrotestosterone	521-18-6		6.6	1.4			0.04	-1.398	Matthews et al. (2000)
GST-rtERdef	5α-Dihydrotestosterone	521-18-6		10	3			0.034	-1.469	Matthews et al. (2000)
hERα	5α-Dihydrotestosterone	521-18-6					0.22	0.05	-1.300	Kuiper et al. (1997)
hERα-FP	5α-Dihydrotestosterone	521-18-6	99	*136*				*0.0095*	-2.022	Bolger et al. (1998)
rERβ	5α-Dihydrotestosterone	521-18-6					0.073	0.17	-0.770	Kuiper et al. (1997)
RUC	5α-Dihydrotestosterone	521-18-6					10	0.026	-1.590	Waller et al. (1996)
MUC	5α-Dihydrotestosterone	521-18-6				10				Korach (1979)
RUC	5α-Dihydrotestosterone	521-18-6	99					0.001	-3.000	Fang et al. (2001)
RUC	5β-Dihydrotestosterone	571-22-2	99			100				Blair et al. (2000)
RUC	2,2'-Dihydroxybenzophenone	835-11-0	98			100				Blair et al. (2000)
RUC	2,4-Dihydroxybenzophenone	131-56-6	99	36.5	4.5			0.002	-2.610	Blair et al. (2000)
RUC	4,4'-Dihydroxybenzophenone	611-99-4	99	26	4			0.003	-2.460	Blair et al. (2000)
RUC	4,4'-Dihydroxybenzophenone	611-99-4	97					0.013	-1.886	Perez et al. (1998)
hERα	4,4'-Dihydroxybiphenyl	92-88-6				10				Kuiper et al. (1998) [method a]

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (μM)**	SD of IC_{50}**	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
hERβ	4,4'-Dihydroxybiphenyl	92-88-6						0.03	-1.523	Kuiper et al. (1998) [method a]
MUC	4,4'-Dihydroxybiphenyl	92-88-6	> 98			5				Korach et al. (1988)
MUC	Dihydroxydiethylstilbestrol	7507-01-9		0.334	0.092			0.48	-0.319	Korach et al. (1978)
RUC	6,4'-Dihydroxyflavone	63046-09-3						0.15	-0.820	Fang et al. (2001)
RUC	3,3'-Dihydroxyhexestrol	79199-51-2		0.00585	0.00165			15.37	1.190	Blair et al. (2000)
RUC	2,2'-Dihydroxy-4-methoxybenzophenone	131-53-3	98			100				Blair et al. (2000)
RUC	3-(2,3 Dihydroxypropoxy)-10-ethyl-11-phenyldibenz- [*b,f*]oxepin	85850-89-1						0.07	-1.155	Acton et al. (1983)
RUC	Diisobutyl phthalate	84-69-5	98			1000				Blair et al. (2000)
RUC	Diisodecyl phthalate	26761-40-0	99.6			1000				Zacharewski et al. (1998)
RUC	Diisoheptyl phthalate	41451-28-9	99.6			1000				Zacharewski et al. (1998)
RUC	Diisononyl phthalate	28553-12-0	Technical grade			1000				Blair et al. (2000)
RUC	Diisononyl phthalate	28553-12-0	99.8			1000				Zacharewski et al. (1998)
RUC	11β-[2-(*N-N* -Dimethylamino)-ethoxy]estra-1,3,5 (10)-triene-3,17b-diol							1.6	0.204	Qian and Abul-Hajj (1990)
RUC	3-[2-(Dimethylamino)ethoxy]-11-ethyl-12-phenyl-6*H* -dibenzo[*b,f*]thioctin	85850-79-9						1.1	0.041	Acton et al. (1983)
RUC	3-[2-(Dimethylamino)ethoxy]-10-ethyl-11-phenyldibenz- [*b,f*] oxepin	85850-76-6						0.02	-1.699	Acton et al. (1983)
RUC	7-[2-(Dimethylamino)ethoxy]-11-ethyl-10-phenyldibenz- [*b,f*]thiepin	85850-77-7						0.12	-0.921	Acton et al. (1983)
RUC	11β-[3-(*N-N* '-Dimethylamino)-propoxy]estra-1,3,5 (10)-triene-3,17β-diol	130043-38-8						2.6	0.415	Qian and Abul-Hajj (1990)
RUC	α,α-Dimethyl-β-ethylallenolic acid	15372-37-9		0.095	0.005			0.95	-0.020	Blair et al. (2000)
RUC	2,6-Dimethylhexestrol	334707-28-7		0.007	0.00173			12.84	1.110	Blair et al. (2000)
RUC	1,6-Dimethylnaphthalene	575-43-9	99			100				Blair et al. (2000)
RUC	Dimethyl phthalate	131-11-3	99			1000				Blair et al. (2000)
RBC	α,α-Dimethylstilbestrol	552-80-7						129	2.111	Korenman (1970)
RUC	α,α-Dimethylstilbestrol	552-80-7		0.0062	0.0013			14.50	1.160	Blair et al. (2000)
MCF-7 cells	Dimethyl sulfoxide	67-68-5				5				Arcaro et al. (1999)
hERα	5,11-*trans* -Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							222	2.346	Meyers et al. (1999)
hERβ	5,11-*trans* -Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							254	2.400	Meyers et al. (1999)
hERα	(5*R* ,11*R*)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							24	1.380	Meyers et al. (1999)
hERβ	(5*R* ,11*R*)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							76	1.880	Meyers et al. (1999)
hERα	(5*S* ,11*S*)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							9.3	0.968	Meyers et al. (1999)
hERβ	(5*S* ,11*S*)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							75	1.880	Meyers et al. (1999)
RUC	Di-*n* -octyl phthalate	117-84-0	98			1000				Blair et al. (2000)
RUC	Di-*n* -octyl phthalate	117-84-0	98.7			1,000				Zacharewski et al. (1998)
RUC	Diphenolic acid	126-00-1	95	120	30			0.0007	-3.130	Blair et al. (2000)
RUC	*trans, trans* -1,4-Diphenyl-1,3-butadiene	886-65-7	98			100				Blair et al. (2000)
RUC	4-[1,2-(Diphenyl-1-butenyl)]phenol acetate	100808-55-7						21	1.322	Jordan et al. (1986)
RUC	2,3-Diphenylindenone-1							0.0095	-2.022	Anstead et al. (1989)
RUC	4-[1-(Diphenylmethylene)-propyl]phenol acetate	82333-68-4						2	0.301	Jordan et al. (1986)
RUC	1,3-Diphenyltetramethyldisiloxane	56-33-7						0.0007	-3.155	Fang et al. (2001)
hERα	5,11-*trans* -Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol							33.6	1.526	Meyers et al. (1999)
hERβ	5,11-*trans* -Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol							92.3	1.970	Meyers et al. (1999)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (µM)**	SD of IC_{50}**	HDT (µM)	Ki (µM)**	RBA***	log RBA***	Reference
hERα	(5*R* ,11*R*)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol							5.2	0.716	Meyers et al. (1999)
hERβ	(5*R* ,11*R*)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol							26	1.410	Meyers et al. (1999)
hERα	(5*S* ,11*S*)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol							1.6	0.204	Meyers et al. (1999)
hERβ	(5*S* ,11*S*)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol							5.1	0.710	Meyers et al. (1999)
RUC	4-Dodecylphenol	104-43-8	99.7	4.85	1.95			0.019	-1.730	Blair et al. (2000)
RUC	Doisynoestrol	15372-34-6		49	14			0.002	-2.740	Blair et al. (2000)
RUC	Dopamine	51-61-6	99			100				Blair et al. (2000)
RUC	Droloxifene	82413-20-5		0.0059	0.0031			15.24	1.180	Blair et al. (2000)
MCF-7 cells	Droloxifene	82413-20-5						0.2	-0.699	Stoessel and Leclerq (1986)
MCF-7 cytosol	Droloxifene	82413-20-5						2.5	0.398	Stoessel and Leclerq (1986)
hERα-FP	Empenthrin	54406-48-3	> 93			10				Saito et al. (2000)
hERα	α-Endosulfan	959-98-8	95 - 99			10				Arcaro et al. (2000)
GST-aERdef	α-Endosulfan	959-98-8				*100*				Matthews et al. (2000)
GST-cERdef	α-Endosulfan	959-98-8				100				Matthews et al. (2000)
GST-hERαdef	α-Endosulfan	959-98-8				*100*				Matthews et al. (2000)
GST-mERαdef	α-Endosulfan	959-98-8				*100*				Matthews et al. (2000)
GST-rtERdef	α-Endosulfan	959-98-8		28	14			0.012	-1.921	Matthews et al. (2000)
hERα	α,β-Endosulfan	115-29-7				10				Kuiper et al. (1998) [method a]
hERβ	α,β-Endosulfan	115-29-7				10				Kuiper et al. (1998) [method a]
MUC	α,β-Endosulfan	115-29-7	98 (a=78%, b=20%)			5				Shelby et al. (1996)
RBC	α,β-Endosulfan	115-29-7	99			100				Andersen et al. (1999)
RUC	α,β-Endosulfan	115-29-7	99			1000				Blair et al. (2000)
RUC	α,β-Endosulfan	115-29-7					599.79	0.00044	-3.360	Waller et al. (1996)
GST-mERαdef	β-Endosulfan	33213-65-9				100				Matthews et al. (2000)
hERα	β-Endosulfan	33213-65-9	95 - 99			10				Arcaro et al. (2000)
GST-aERdef	β-Endosulfan	33213-65-9				100				Matthews et al. (2000)
GST-cERdef	β-Endosulfan	33213-65-9				100				Matthews et al. (2000)
GST-hERαdef	β-Endosulfan	33213-65-9				100				Matthews et al. (2000)
GST-rtERdef	β-Endosulfan	33213-65-9				*100*				Matthews et al. (2000)
MCF-7 cytosol	β-Endosulfan	33213-65-9	Technical grade	631	88			0.00024	-3.620	Soto et al. (1995)
RBC	16-Epiestriol	547-81-9						44	1.643	Korenman (1969)
hERα	17-Epiestriol	1228-72-4						29	1.462	Kuiper et al. (1998) [method a]
hERβ	17-Epiestriol	1228-72-4						80	1.903	Kuiper et al. (1998) [method a]
RUC	Epitestosterone	481-30-1	99.9			600				Blair et al. (2000)
RBC	Equilenin	517-09-9						8	0.903	Korenman (1969)
RBC	Equilin	474-86-2						24	1.380	Korenman (1969)
hERα	Equol	531-95-3		1.5				0.33	-0.48	Morito et al. (2001)
hERβ	Equol	531-95-3		0.0085				58.8	1.77	Morito et al. (2001)
RUC	Equol	531-95-3						0.15	-0.820	Fang et al. (2001)
RBC	*erythro* -MEA	20576-52-7						135	2.130	Korenman (1970)
RBC	16α-Estradiol	1090-04-6						66	1.820	Korenman (1969)
MCF-7 cytosol	16α-Estradiol	1090-04-6						35	1.544	Brooks et al. (1987)
MCF-7 cytosol	16α-Estradiol	1090-04-6						0.8	-0.097	VanderKuur et al. (1993)
hERα	17α-Estradiol	57-91-0					0.0002	58	1.760	Kuiper et al. (1997)
hERα	17α-Estradiol	57-91-0						7	0.845	Kuiper et al. (1998) [method a]
hERβ	17α-Estradiol	57-91-0						2	0.301	Kuiper et al. (1998) [method a]

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (µM)**	SD of IC_{50}**	HDT (µM)	Ki (µM)**	RBA***	log RBA***	Reference
MCF-7 cytosol	17α-Estradiol	57-91-0						0.22	-0.658	VanderKuur et al. (1993)
MUC	17α-Estradiol	57-91-0						1000	3.000	Korach (1979)
RBC	17α-Estradiol	57-91-0						49	1.690	Korenman (1969)
rERβ	17α-Estradiol	57-91-0					0.0012	11	1.041	Kuiper et al. (1997)
RUC	17α-Estradiol	57-91-0	99	0.0293	0.008			3.07	0.490	Blair et al. (2000)
RUC	17α-Estradiol	57-91-0		0.01				50	1.699	Elsby et al. (2000)
GST-hERαdef	17β-Estradiol	50-28-2		0.0055	0.0012			100	2.00	Fertuck et al. (2001)
hERα	17β-Estradiol	50-28-2		*0.0006*				*100*	*2.000*	Arcaro et al. (1999)
hERα	17β-Estradiol	50-28-2		0.0005				100	2.000	Arcaro et al. (2000)
hERα	17β-Estradiol	50-28-2	> 97	*0.004*				100	2.000	Gaido et al. (1999)
hERα	17β-Estradiol	50-28-2		0.001				100.0	2.000	Klotz et al. (1996)
hERα	17β-Estradiol	50-28-2						100	2.000	Kraichely et al. (2000)
hERα	17β-Estradiol	50-28-2		0.00021			0.00013	100	2.000	Kuiper et al. (1997)
hERα	17β-Estradiol	50-28-2						100	2.000	Kuiper et al. (1998) [method a]
hERα	17β-Estradiol	50-28-2		0.00093				100	2.000	Kuiper et al. (1998) [method b]
hERα	17β-Estradiol	50-28-2						100	2.000	Meyers et al. (1999)
hERα	17β-Estradiol	50-28-2		0.005				100	2.00	Morito et al. (2001)
hERα	17β-Estradiol	50-28-2					0.0003	100	2.000	Sun et al. (1999)
hERα	17β-Estradiol	50-28-2		0.00059				100	2.000	Vakharia and Gierthy (1999)
hERα	17β-Estradiol	50-28-2		0.001				100	2.000	Vakharia and Gierthy (2000)
hERα-FP	17β-Estradiol	50-28-2	98	0.013				100	2.000	Bolger et al. (1998)
hERα-FP	17β-Estradiol	50-28-2		0.0195	0.0018			100	2.000	Hanioka et al. (1999)
hERα-FP	17β-Estradiol	50-28-2		0.01				100	2.000	Hashimoto et al. (2000)
hERα-FP	17β-Estradiol	50-28-2		0.013	0.0007			100	2.000	Nikov et al. (2000)
hERα-FP	17β-Estradiol	50-28-2		0.013	0.0007			100	2.000	Nikov et al. (2001)
hERα-FP	17β-Estradiol	50-28-2		0.0056				100	2.000	Parker et al. (2000)
hERα-FP	17β-Estradiol	50-28-2		*0.04*				*100*	2.000	Saito et al. (2000)
hERβ	17β-Estradiol	50-28-2		*0.005*				*100*	*2.000*	Arcaro et al. (1999)
hERβ	17β-Estradiol	50-28-2		0.0056	0.0011			100	2.00	Fertuck et al. (2001)
hERβ	17β-Estradiol	50-28-2	> 97					100	2.000	Gaido et al. (1999)
hERβ	17β-Estradiol	50-28-2						100	2.000	Kraichely et al. (2000)
hERβ	17β-Estradiol	50-28-2						100	2.000	Kuiper et al. (1998) [method a]
hERβ	17β-Estradiol	50-28-2						100	2.000	Kuiper et al. (1998) [method a]
hERβ	17β-Estradiol	50-28-2		0.00106				100	2.000	Kuiper et al. (1998) [method b]
hERβ	17β-Estradiol	50-28-2						100	2.000	Meyers et al. (1999)
hERβ	17β-Estradiol	50-28-2		0.005				100	2.00	Morito et al. (2001)
hERβ	17β-Estradiol	50-28-2					0.0009	100	2.000	Sun et al. (1999)
MCF-7 cells	17β-Estradiol	50-28-2						100	2.000	Arcaro et al. (1999)
MCF-7 cells	17β-Estradiol	50-28-2						100	2.000	Brooks et al. (1987)
MCF-7 cells	17β-Estradiol	50-28-2		*0.0004*				*100*	2.000	Lascombe et al. (2000)
MCF-7 cells	17β-Estradiol	50-28-2						100	2.000	Nagel et al. (1997)
MCF-7 cytosol	17β-Estradiol	50-28-2						100	2.000	Brooks et al. (1987)
MCF-7 cytosol	17β-Estradiol	50-28-2		*0.0009*				100	2.000	Dodge et al. (1996)
MCF-7 cytosol	17β-Estradiol	50-28-2		1.3	0.8			100	2.000	Kramer et al. (1997)
MCF-7 cytosol	17β-Estradiol	50-28-2						100	2.000	Palomino et al. (1994)
MCF-7 cytosol	17β-Estradiol	50-28-2		0.0015	0.0004			100	2.000	Soto et al. (1995)
MCF-7 cytosol	17β-Estradiol	50-28-2						100	2.000	VanderKuur et al. (1993)
MUC	17β-Estradiol	50-28-2		0.0021				100	2.000	Connor et al. (1997)
MUC	17β-Estradiol	50-28-2		0.015				100	2.000	Fielden et al. (1997)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (μM)**	SD of IC_{50}**	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
MUC	17β-Estradiol	50-28-2		0.001				100	2.000	Korach (1979)
MUC	17β-Estradiol	50-28-2		0.0016	0.0005			100	2.000	Korach et al. (1978)
MUC	17β-Estradiol	50-28-2		1.6	0.5			100	2.000	Korach et al. (1985)
MUC	17β-Estradiol	50-28-2		0.0010				100	2.000	Korach et al. (1988)
MUC	17β-Estradiol	50-28-2						100	2.000	Korach et al. (1989)
MUC	17β-Estradiol	50-28-2		0.003	0.0002			100	2.000	Matthews et al. (2001)
MUC	17β-Estradiol	50-28-2		*0.0032*				100	2.000	Ramamoorthy et al. (1997a)
MUC	17β-Estradiol	50-28-2		0.011				100	2.000	Ramamoorthy et al. (1997b)
MUC	17β-Estradiol	50-28-2	> 99	*0.0018*				*100*	*2.000*	Shelby et al. (1996)
RBC	17β-Estradiol	50-28-2						100	2.000	Korenman (1969)
rERβ	17β-Estradiol	50-28-2		0.00013			0.00012	100	2.000	Kuiper et al. (1997)
RUC	17β-Estradiol	50-28-2						100	2.000	Acton et al. (1983)
RUC	17β-Estradiol	50-28-2		*0.003*				*100*	2.000	Ashby et al. (1999)
RUC	17β-Estradiol	50-28-2						100	2.000	Gabbard and Segaloff (1983)
RUC	17β-Estradiol	50-28-2		*0.008*				*100*	2.000	Jaimez et al. (2000)
RUC	17β-Estradiol	50-28-2						100	2.000	Jordan et al. (1986)
RUC	17β-Estradiol	50-28-2		0.0011			0.0004	100	2.000	Laws et al. (2000)
RUC	17β-Estradiol	50-28-2		*0.001*				100	2.000	Olea et al. (1996)
RUC	17β-Estradiol	50-28-2						100	2.000	Qian and Abul-Hajj (1990)
RUC	17β-Estradiol	50-28-2					0.0026	100	2.000	Waller et al. (1996)
GST-aERdef	17β-Estradiol	50-28-2		0.0025	0.0013			100	2.000	Matthews and Zacharewski (2000)
GST-aERdef	17β-Estradiol	50-28-2		0.0031	0.0005			100	2.000	Matthews et al. (2000)
GST-cERdef	17β-Estradiol	50-28-2		0.0032	0.0005			100	2.000	Matthews et al. (2000)
GST-hERαdef	17β-Estradiol	50-28-2		0.0024	0.001			100	2.000	Matthews and Zacharewski (2000)
GST-hERαdef	17β-Estradiol	50-28-2		0.0029	0.0005			100	2.000	Matthews et al. (2000)
GST-mERαdef	17β-Estradiol	50-28-2		0.0027	0.0004			100	2.000	Matthews et al. (2000)
GST-rtERdef	17β-Estradiol	50-28-2		0.0031	0.0006			100	2.000	Matthews and Zacharewski (2000)
GST-rtERdef	17β-Estradiol	50-28-2		0.0033	0.0005			100	2.000	Matthews et al. (2000)
RBC	17β-Estradiol	50-28-2	99.4	0.00002				100	2.000	Andersen et al. (1999)
RUC	17β-Estradiol	50-28-2		0.00625				100	2.000	Allen et al. (1980)
RUC	17β-Estradiol	50-28-2		0.000899	0.000027			100	2.000	Blair et al. (2000)
RUC	17β-Estradiol	50-28-2		0.014				100	2.000	Connor et al. (1997)
RUC	17β-Estradiol	50-28-2		0.005				*100*	2.000	Elsby et al. (2000)
RUC	17β-Estradiol	50-28-2	98	0.003				100	2.000	Laws et al. (1996)
RUC	17β-Estradiol	50-28-2		*0.0016*				100	2.000	Leibl and Spona (1982)
RUC	17β-Estradiol	50-28-2		0.00146			0.00005	100	2.000	McBlain (1987)
RUC	17β-Estradiol	50-28-2		*0.0009*				100	2.000	Nelson (1974)
RUC	17β-Estradiol	50-28-2						100	2.000	Perez et al. (1998)
RUC	17β-Estradiol	50-28-2		0.0013				100	2.000	Zacharewski et al. (1998)
MCF-7 cytosol	9β-Estradiol							0.7	-0.155	Palomino et al. (1994)
RBC	Estradiol 17-acetate							29	1.462	Korenman (1969)
RBC	17β-Estradiol 3-acetate	4245-41-4						97	1.987	Korenman (1969)
GST-aERdef	Estradiol 3-benzoate	50-50-0		0.024	0.003			13	1.114	Matthews et al. (2000)
GST-cERdef	Estradiol 3-benzoate	50-50-0		0.022	0.001			15	1.176	Matthews et al. (2000)
GST-hERαdef	Estradiol 3-benzoate	50-50-0		0.028	0.005			10	1.000	Matthews et al. (2000)
GST-mERαdef	Estradiol 3-benzoate	50-50-0		0.023	0.002			12	1.079	Matthews et al. (2000)
GST-rtERdef	Estradiol 3-benzoate	50-50-0		0.0037	0.0005			9	0.954	Matthews et al. (2000)
RBC	Estradiol diacetate	3434-88-6						11	1.041	Korenman (1969)
RBC	17β-Estradiol 3-methyl ether	1035-77-4						3	0.477	Korenman (1969)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (μM)**	SD of IC_{50}**	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
RUC	17β-Estradiol 3-methyl ether	1035-77-4						0.7	-0.155	Gabbard and Segaloff (1983)
MCF-7 cells	9-Estratetraene-3,17β-diol	791-69-5						37	1.568	Stoessel and Leclerq (1986)
MCF-7 cytosol	9-Estratetraene-3,17β-diol	791-69-5						80	1.903	Stoessel and Leclerq (1986)
RBC	Estra-1,3,5(10),6-tetraen-17-one, 3-hydroxy-							10	1.000	Korenman (1969)
MCF-7 cells	Estra-1,3,5(10)-triene-3,17β-diol, 14,15α-epoxy-	79581-12-7						10	1.000	Stoessel and Leclerq (1986)
MCF-7 cytosol	Estra-1,3,5(10)-triene-3,17β-diol, 14,15α-epoxy-	79581-12-7						5	0.699	Stoessel and Leclerq (1986)
MCF-7 cytosol	Estra-1,3,5(10)-triene-3,17β-diol, 14β,15β-epoxy-	79645-49-1				0.1				Stoessel and Leclerq (1986)
MCF-7 cells	Estra-1,3,5(10)-triene-3,17β-diol, 14β,15β-epoxy-	79645-49-1						0.08	-1.097	Stoessel and Leclerq (1986)
MCF-7 cells	Estra-1,3,5,(10),trien-3,14,17β-triol	16288-09-8						1.5	0.176	Stoessel and Leclerq (1986)
MCF-7 cytosol	Estra-1,3,5,(10),trien-3,14,17β-triol	16288-09-8						2	0.301	Stoessel and Leclerq (1986)
RUC	Estratriene-3,6α,17β-triol	1229-24-9		0.127	0.043			0.71	-0.150	Blair et al. (2000)
GST-aERdef	Estriol	50-27-1		0.01	0.001			30	1.477	Matthews et al. (2000)
GST-cERdef	Estriol	50-27-1		0.029	0.001			11	1.041	Matthews et al. (2000)
GST-hERαdef	Estriol	50-27-1		0.01	0.003			28	1.447	Matthews et al. (2000)
GST-mERαdef	Estriol	50-27-1		0.021	0.005			13	1.114	Matthews et al. (2000)
GST-rtERdef	Estriol	50-27-1		0.09	0.006			3.7	0.568	Matthews et al. (2000)
hERα	Estriol	50-27-1					0.0014	14	1.146	Kuiper et al. (1997)
MCF-7 cells	Estriol	50-27-1						20	1.301	Stoessel and Leclerq (1986)
MCF-7 cytosol	Estriol	50-27-1						13	1.114	Brooks et al. (1987)
MCF-7 cytosol	Estriol	50-27-1						18	1.255	Stoessel and Leclerq (1986)
MCF-7 cytosol	Estriol	50-27-1						0.17	-0.770	VanderKuur et al. (1993)
MUC	Estriol	50-27-1						100	2.000	Korach (1979)
RBC	Estriol	50-27-1						16	1.204	Korenman (1969)
rERβ	Estriol	50-27-1					0.0007	21	1.320	Kuiper et al. (1997)
RUC	Estriol	50-27-1					0.014	19	1.279	Waller et al. (1996)
RUC	Estriol	50-27-1	99	0.00925	0.00175			9.719	0.990	Blair et al. (2000)
GST-aERdef	Estrone	53-16-7		0.0051	0.0001			60	1.778	Matthews et al. (2000)
GST-cERdef	Estrone	53-16-7		0.0064	0.0001			50	1.699	Matthews et al. (2000)
GST-hERαdef	Estrone	53-16-7		0.0065	0.0003			45	1.653	Matthews et al. (2000)
GST-mERαdef	Estrone	53-16-7		0.0095	0.0008			28	1.447	Matthews et al. (2000)
GST-rtERdef	Estrone	53-16-7		0.024	0.002			14	1.146	Matthews et al. (2000)
hERα	Estrone	53-16-7					0.0003	60	1.778	Kuiper et al. (1997)
hERα-FP	Estrone	53-16-7	98	0.626				2.1	0.322	Bolger et al. (1998)
MCF-7 cytosol	Estrone	53-16-7						0.22	-0.658	VanderKuur et al. (1993)
MUC	Estrone	53-16-7						100	2.000	Korach (1979)
rERβ	Estrone	53-16-7					0.0004	37	1.568	Kuiper et al. (1997)
RUC	Estrone	53-16-7	99	0.0123	0.0032			7.31	0.860	Blair et al. (2000)
RUC	Estrone	53-16-7		0.01				*50*	1.699	Elsby et al. (2000)
RUC	Estrone	53-16-7						46	1.663	Gabbard and Segaloff (1983)
RUC	Estrone	53-16-7					0.0044	59	1.771	Waller et al. (1996)
RBC	Estrone	53-16-7						66	1.820	Korenman (1969)
MCF-7 cells	Estrone	53-16-7						19	1.279	Stoessel and Leclerq (1986)
MCF-7 cytosol	Estrone	53-16-7						13	1.114	Brooks et al. (1987)
MCF-7 cytosol	Estrone	53-16-7						15	1.176	Stoessel and Leclerq (1986)
RBC	Estrone 3-acetate	901-93-9						191	2.281	Korenman (1969)
RUC	Estrone 3-methyl ether	1624-62-0				1				Gabbard and Segaloff (1983)
hERa	Estrone 3-sulfate	481-97-0				100				Kuiper et al. (1997)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (μM)**	SD of IC_{50}**	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
rERβ	Estrone 3-sulfate	481-97-0				100				Kuiper et al. (1997)
GST-aERdef	17α-Ethinyl estradiol	57-63-6		0.0022	0.0001			139	2.143	Matthews et al. (2000)
GST-cERdef	17α-Ethinyl estradiol	57-63-6		0.0019	0.0001			171	2.233	Matthews et al. (2000)
GST-hERαdef	17α-Ethinyl estradiol	57-63-6		0.0023	0.0001			127	2.104	Matthews et al. (2000)
GST-mERαdef	17α-Ethinyl estradiol	57-63-6		0.0022	0.0007			118	2.072	Matthews et al. (2000)
GST-rtERdef	17α-Ethinyl estradiol	57-63-6		0.0031	0.0009			108	2.033	Matthews et al. (2000)
RBC	17α-Ethinyl estradiol	57-63-6						191	2.281	Korenman (1969)
RUC	17α-Ethinyl estradiol	57-63-6	98	0.004				156	2.180	Allen et al. (1980)
RUC	17α-Ethinyl estradiol	57-63-6						190	2.279	Fang et al. (2001)
RUC	17α-Ethinyl estradiol	57-63-6		0.0011			0.0004	100	2.000	Laws et al. (2000)
RUC	17α-Ethinyl estradiol	57-63-6					0.0002999	867	2.938	Waller et al. (1996)
RUC	17β-Ethinyl estradiol	4717-38-8		0.000473	0.00006			190.063	2.280	Blair et al. (2000)
RUC	Ethyl cinnamate	103-36-6	99.1			1000				Blair et al. (2000)
RUC	3-Ethyl-6,4'-dihydroxy-2-phenylindene							16	1.204	Anstead et al. (1989)
RUC	2-Ethylhexyl paraben	5153-25-3	99	4 95	0.05			0.018	-1.740	Blair et al. (2000)
RUC	4-Ethyl-7-hydroxy-3-(4-methoxyphenyl)-2*H* -1-benzopyran-2-one	5219-17-0						0.9	-0.460	Fang et al. (2001)
RUC	3-[(10-Ethyl-11-*p* -hydroxyphenyl)dibenzo-[*b,f*]oxepin-3-yl)oxy]-1,2-propanediol, hydrate (4:1)	85850-93-7						0.92	-0.036	Acton et al. (1983)
RUC	3-[(10-Ethyl-11-*p* -hydroxyphenyl)dibenzo-[*b,f*]thiepin-3-yl)oxy]-1,2-propanediol	85850-94-8						11.0	1.041	Acton et al. (1983)
RUC	3-[(11-Ethyl-12-(*p* -hydroxyphenyl)-6-*H*-dibenzo-[*b,f*]thiocin-3-yl)oxy]-, hemihydrate 1,2-propanediol	85864-54-6						5.0	0.699	Acton et al. (1983)
RUC	3-[(6-Ethyl-5-(*p* -hydroxyphenyl)-11,12-dihydrodibenzo-[*a,e*]cycloocten-2-yl)oxy] 1,2-propanediol	85850-95-9						9.1	0.959	Acton et al. (1983)
RUC	3-Ethyl-4'-hydroxy-2-phenylindene							2.3	0.362	Anstead et al. (1989)
RUC	3-Ethyl-6-hydroxy 2-phenylindene							0.58	-0.237	Anstead et al. (1989)
RUC	3-Ethyl-4'-hydroxy 2-phenylindenone-1							4.6	0.663	Anstead et al. (1989)
RUC	3-Ethyl-6-hydroxy 2-phenylindenone-1							1.2	0.079	Anstead et al. (1989)
RBC	3-Ethyl-4-(*p* -methoxyphenyl)-2-methyl-3-cyclohexene-1-carboxylic acid	1755-52-8						0.75	-0.125	Korenman (1969)
RUC	Ethyl paraben	120-47-8	99	150	10			0.0006	-3.220	Blair et al. (2000)
RUC	2-Ethylphenol	90-00-6	99			1000				Blair et al. (2000)
RUC	3-Ethylphenol	620-17-7	80	660	76			0.00014	-3 87	Blair et al. (2000)
RUC	4-Ethylphenol	123-07-9	99	1340	40			0.00007	-4.170	Blair et al. (2000)
RUC	3-[(10-Ethyl-11-phenyldibenzo[*b,f*]thiepin-3-yl)oxy]-1,2-propanediol, complexed with isopropyl alcohol 2:1	85850-90-4						0.65	-0.187	Acton et al. (1983)
RUC	3-[(11-Ethyl-12-phenyl-6*H* -dibenzo[*b,f*]thiocin-3-yl)oxy]-1,2-propanediol, hydrate (4:1)	85850-92-6						0.02	-1.699	Acton et al. (1983)
RUC	3-[6-Ethyl-5-phenyl-11,12-dihydrodibenzo[*a,e*]cycloocten-2-yl)oxy]-1,2-propanediol	85850-91-5						0.12	-0.921	Acton et al. (1983)
RUC	Eugenol	97-53-0	99.2			1000				Blair et al. (2000)
hERα-FP	Fenvalerate	51630-58-1	> 93			10				Saito et al. (2000)
RUC	Fisetin	528-48-3						0.0045	2.350	Fang et al. (2001)
RUC	Flavanone	17002-31-2				100				Fang et al. (2001)
hERα	Flavone	525-82-6				10				Kuiper et al. (1998) [method a]
hERβ	Flavone	525-82-6				10				Kuiper et al. (1998) [method a]
RUC	Flavone	525-82-6				100				Fang et al. (2001)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (μM)**	SD of IC_{50}**	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
MCF-7 cells	Fluoranthene	206-44-0				5				Arcaro et al. (1999)
MCF-7 cells	Fluorene	86-73-7				5				Arcaro et al. (1999)
MCF-7 cytosol	2-Fluoroestratrien-17β-ol	101772-22-9						2	0.301	Brooks et al. (1987)
MCF-7 cytosol	4-Fluoroestratrien-17β-ol	96607-54-4						8	0.903	Brooks et al. (1987)
RUC	2-(2-Fluorophenyl)-3-phenyl-6-hydroxyindene							49	1.690	Anstead et al. (1990)
RUC	Fluorotamoxifen	73617-96-6				10				Allen et al. (1980)
RUC	Folic acid	59-30-3				100				Blair et al. (2000)
hERα	Formononetin	485-72-3				10				Kuiper et al. (1998) [method a]
hERβ	Formononetin	485-72-3				10				Kuiper et al. (1998) [method a]
RUC	Formononetin	485-72-3						0.0013	-2.890	Fang et al. (2001)
RUC	Furfural	98-01-1	99.4			1000				Blair et al. (2000)
GST-aERdef	Genistein	446-72-0		0.24	0.01			1.3	0.114	Matthews et al. (2000)
GST-cERdef	Genistein	446-72-0		0.41	0.05			0.78	-0.108	Matthews et al. (2000)
GST-hERαdef	Genistein	446-72-0		0.063	0.07			0.46	-0.337	Matthews et al. (2000)
GST-mERαdef	Genistein	446-72-0		0.81	0.04			0.33	-0.481	Matthews et al. (2000)
GST-rtERdef	Genistein	446-72-0		0.75	0.08			0.44	-0.357	Matthews et al. (2000)
hERα	Genistein	446-72-0					0.0026	5	0.699	Kuiper et al. (1997)
hERα	Genistein	446-72-0						4	0.602	Kuiper et al. (1998) [method a]
hERα	Genistein	446-72-0		0.145				0.7	-0.155	Kuiper et al. (1998) [method b]
hERα	Genistein	446-72-0		0.7				0.71	-0.15	Morito et al. (2001)
hERα-FP	Genistein	446-72-0		0.825	0.002			1.6	0.204	Nikov et al. (2000)
hERβ	Genistein	446-72-0						87	1.940	Kuiper et al. (1998) [method a]
hERβ	Genistein	446-72-0		0.0084				13	1.114	Kuiper et al. (1998) [method b]
hERβ	Genistein	446-72-0		0.011				45.5	1.66	Morito et al. (2001)
MCF-7 cytosol	Genistein	446-72-0		*0.045*				2	0.301	Dodge et al. (1996)
MCF-7 cytosol	Genistein	446-72-0		*0.23*				*2.61*	0.417	Miodini et al. (1999)
rERβ	Genistein	446-72-0					0.0003	36	1.556	Kuiper et al. (1997)
RUC	Genistein	446-72-0						0.45	-0.350	Fang et al. (2001)
RUC	Genistein	446-72-0					0.39	0.67	-0.180	Waller et al. (1996)
hERβ	Genistin	529-59-9		37				0.014	-1.87	Morito et al. (2001)
RUC	Genistin	529-59-9				100				Fang et al. (2001)
hERα-FP	Glyceollin	66241-09-6		6	0.6			0.22	-0.658	Nikov et al. (2000)
hERα	Glycitein	40957-83-3		32				0.016	-1.81	Morito et al. (2001)
hERβ	Glycitein	40957-83-3		0.55				0.91	-0.04	Morito et al. (2001)
hERβ	Glycitin			650				0.0008	-3.10	Morito et al. (2001)
RUC	Heptachlor	76-44-8	99.5			100				Blair et al. (2000)
GST-aERdef	2,2',3,3',4',5',6'-Heptachlorobiphenyl	52663-70-4				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3,3',4',5',6'-Heptachlorobiphenyl	52663-70-4				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,3',4',5',6'-Heptachlorobiphenyl	52663-70-4		10				0.031	-1.509	Matthews and Zacharewski (2000)
GST-aERdef	2,2',3,3',4,5,6,-Heptachlorobiphenyl	68194-16-1				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3,3',4,5,6,-Heptachlorobiphenyl	68194-16-1				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,3',4,5,6,-Heptachlorobiphenyl	68194-16-1		10				0.031	-1.509	Matthews and Zacharewski (2000)
GST-aERdef	2,2',3,3',5,5',6-Heptachlorobiphenyl	52663-64-6				10				Matthews and Zacharewski (2000)
GST-hERadef	2,2',3,3',5,5',6-Heptachlorobiphenyl	52663-64-6				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,3',5,5',6-Heptachlorobiphenyl	52663-64-6				10				Matthews and Zacharewski (2000)
GST-aERdef	2,2',3,4,4',5',6-Heptachlorobiphenyl	52663-69-1				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3,4,4',5',6-Heptachlorobiphenyl	52663-69-1				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,4,4',5',6-Heptachlorobiphenyl	52663-69-1				10				Matthews and Zacharewski (2000)
GST-aERdef	2,2',3,4,4',6,6'-Heptachlorobiphenyl	74472-48-3		10				0.025	-1.602	Matthews and Zacharewski (2000)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (μM)**	SD of IC_{50}**	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
GST-hERαdef	2,2',3,4,4',6,6'-Heptachlorobiphenyl	74472-48-3		10				0.024	-1.620	Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,4,4',6,6'-Heptachlorobiphenyl	74472-48-3		0.4	0.1			0.78	-0.111	Matthews and Zacharewski (2000)
GST-aERdef	2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0				10				Matthews and Zacharewski (2000)
GST-aERdef	2,2',3,4',5,6,6'-Heptachlorobiphenyl	74487-85-7		10				0.025	-1.602	Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3,4',5,6,6'-Heptachlorobiphenyl	74487-85-7		10				0.024	-1.620	Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,4',5,6,6'-Heptachlorobiphenyl	74487-85-7		1.3	1.2			0.24	-0.623	Matthews and Zacharewski (2000)
GST-aERdef	2,3,3',4,4',5,6-Heptachlorobiphenyl	41411-64-7				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,3,3',4,4',5,6-Heptachlorobiphenyl	41411-64-7				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,3,3',4,4',5,6-Heptachlorobiphenyl	41411-64-7				10				Matthews and Zacharewski (2000)
GST-aERdef	2,3,3',4',5,5',6-Heptachlorobiphenyl	69782-91-8				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,3,3',4',5,5',6-Heptachlorobiphenyl	69782-91-8				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,3,3',4',5,5',6-Heptachlorobiphenyl	69782-91-8				10				Matthews and Zacharewski (2000)
hERα	2,2',3,3',4',5,5'-Heptachloro-4-biphenylol	158076-64-3	> 98					0.1	-1.000	Kuiper et al. (1998) [method a]
hERβ	2,2',3,3',4',5,5'-Heptachloro-4-biphenylol	158076-64-3	> 98					0.1	-1.000	Kuiper et al. (1998) [method a]
GST-aERdef	2,2',3,3',4',5,5'-Heptachloro-4-biphenylol	158076-64-3				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3,3',4',5,5'-Heptachloro-4-biphenylol	158076-64-3				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,3',4',5,5'-Heptachloro-4-biphenylol	158076-64-3				10				Matthews and Zacharewski (2000)
hERα	2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8	> 98					0.09	-1.046	Kuiper et al. (1998) [method a]
hERα	2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8	> 98					0.09	-1.046	Kuiper et al. (1998) [method a]
hERβ	2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8	> 98					0.1	-1.000	Kuiper et al. (1998) [method a]
hERβ	2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8	> 98					0.1	-1.000	Kuiper et al. (1998) [method a]
GST-aERdef	2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8				10				Matthews and Zacharewski (2000)
hERα	2,2',3,4',5,5',6-Heptachloro-4-biphenylol	158076-68-7	> 98					0.1	-1.000	Kuiper et al. (1998) [method a]
hERβ	2,2',3,4',5,5',6-Heptachloro-4-biphenylol	158076-68-7	> 98					0.1	-1.000	Kuiper et al. (1998) [method a]
RUC	Heptanal	111-71-7	92.9			10000				Blair et al. (2000)
RUC	4-(Heptyloxy)phenol	13037-86-0	97	67.5	7.5			0.0013	-2.880	Blair et al. (2000)
RUC	Heptyl 4-paraben	1085-12-7	97	11	1			0.008	-2.090	Blair et al. (2000)
RUC	Hesperetin	520-33-2				100				Fang et al. (2001)
RUC	Hexachlorobenzene	118-74-1				1000				Blair et al. (2000)
GST-aERdef	2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3				10				Matthews and Zacharewski (2000)
GST-aERdef	2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2				10				Matthews and Zacharewski (2000)
GST-aERdef	2,2',3,4',5',6-Hexachlorobiphenyl	38380-04-0				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3,4',5',6-Hexachlorobiphenyl	38380-04-0				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,4',5',6-Hexachlorobiphenyl	38380-04-0				10				Matthews and Zacharewski (2000)
GST-aERdef	2,2',3,4,5,6'-Hexachlorobiphenyl	68194-15-0				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3,4,5,6'-Hexachlorobiphenyl	68194-15-0				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,4,5,6'-Hexachlorobiphenyl	68194-15-0		10				0.031	-1.509	Matthews and Zacharewski (2000)
GST-aERdef	2,2',3,5,5',6-Hexachlorobiphenyl	52663-63-5				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3,5,5',6-Hexachlorobiphenyl	52663-63-5				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,5,5',6-Hexachlorobiphenyl	52663-63-5				10				Matthews and Zacharewski (2000)
GST-aERdef	2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1				10				Matthews and Zacharewski (2000)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (µM)**	SD of IC_{50}**	HDT (µM)	Ki (µM)**	RBA***	log RBA***	Reference
GST-hERαdef	2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1				10				Matthews and Zacharewski (2000)
hERα	2,2',4,4',6,6'-Hexachlorobiphenyl	33979-03-2	≥ 99%			50				Vakharia and Gierthy (2000)
MUC	2,2',4,4',6,6'-Hexachlorobiphenyl	33979-03-2	> 98	5.6				0.27	-0.569	Fielden et al. (1997)
GST-aERdef	2,3,3',4,4',6-Hexachlorobiphenyl	74472-42-7				10				Matthews and Zacharewski (2000)
GST-hERadef	2,3,3',4,4',6-Hexachlorobiphenyl	74472-42-7				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,3,3',4,4',6-Hexachlorobiphenyl	74472-42-7				10				Matthews and Zacharewski (2000)
GST-aERdef	2,3',4,4',5',6-Hexachlorobiphenyl	59291-65-5				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,3',4,4',5',6-Hexachlorobiphenyl	59291-65-5				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,3',4,4',5',6-Hexachlorobiphenyl	59291-65-5				10				Matthews and Zacharewski (2000)
GST-aERdef	3,3',4,4',5,5'-Hexachlorobiphenyl	32774-16-6				10				Matthews and Zacharewski (2000)
GST-hERαdef	3,3',4,4',5,5'-Hexachlorobiphenyl	32774-16-6				10				Matthews and Zacharewski (2000)
GST-rtERdef	3,3',4,4',5,5'-Hexachlorobiphenyl	32774-16-6				10				Matthews and Zacharewski (2000)
hERα	2,2',3,3',4',5-Hexachloro-4-biphenylol	158076-62-1	> 98					0.07	-1.155	Kuiper et al. (1998) [method a]
hERβ	2,2',3,3',4',5-Hexachloro-4-biphenylol	158076-62-1	> 98					0.06	-1.222	Kuiper et al. (1998) [method a]
GST-aERdef	2,2',3,3',4',5-Hexachloro-4-biphenylol	158076-62-1				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3,3',4',5-hexachloro-4-biphenylol	158076-62-1				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,3',4',5-Hexachloro-4-biphenylol	158076-62-1				10				Matthews and Zacharewski (2000)
GST-aERdef	2,2',3,4',5,5'-Hexachloro-4-biphenylol	145413-90-7				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3,4',5,5'-Hexachloro-4-biphenylol	145413-90-7				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,4',5,5'-Hexachloro-4-biphenylol	145413-90-7				10				Matthews and Zacharewski (2000)
hERα	2,2',3,4',5,5'-Hexachloro-4-biphenylol	145413-90-7	> 98					0.03	-1.523	Kuiper et al. (1998) [method a]
hERβ	2,2',3,4',5,5'-Hexachloro-4-biphenylol	145413-90-7	> 98					0.04	-1.398	Kuiper et al. (1998) [method a]
MCF-7 cytosol	2',3,3',4',5,5'-Hexachloro-4-biphenylol	158076-63-2	> 95	2.8				3.2	0.505	Kramer et al. (1997)
RUC	n-Hexanol	111-27-3	98.9			10000				Blair et al. (2000)
hERα	Hexestrol	84-16-2					0.00006	302	2.480	Kuiper et al. (1997)
MCF-7 cells	Hexestrol	84-16-2						58	1.763	Stoessel and Leclerq (1986)
MCF-7 cytosol	Hexestrol	84-16-2						100	2.000	Stoessel and Leclerq (1986)
RBC	Hexestrol	84-16-2						74	1.869	Korenman (1969)
rERβ	Hexestrol	84-16-2					0.00006	234	2.369	Kuiper et al. (1997)
RUC	Hexestrol	84-16-2	99	0.0003	0			299.67	2.480	Blair et al. (2000)
RUC	Hexestrol	84-16-2						300	2.477	Fang et al. (2001)
RUC	*DL* -Hexestrol	5776-72-7		0.025	0.005			3.60	0.560	Blair et al. (2000)
RUC	Hexestrol monomethyl ether	13026-26-1		0.0096	0.0014			9.37	0.970	Blair et al. (2000)
GST-hERαdef	3-Hydroxybenzo[*b*]naphtho[2,1-d]thiophene			0.3	0.074			1.83	0.26	Fertuck et al. (2001)
hERβ	3-Hydroxybenzo[*b*]naphtho[2,1-d]thiophene			0.22	0.082			2.50	0.40	Fertuck et al. (2001)
GST-hERαdef	2-Hydroxybenzo- [*c*]phenanthrene	22717-94-8		0.25	0.004			2.2	0.34	Fertuck et al. (2001)
hERβ	2-Hydroxybenzo- [*c*]phenanthrene	22717-94-8		0.18	0.1			3.10	0.49	Fertuck et al. (2001)
GST-hERαdef	3-Hydroxybenzo- [*b*]phenanthro[2,3-d]thiophene			0.23	0.01			2.40	0.38	Fertuck et al. (2001)
hERβ	3-Hydroxybenzo- [*b*]phenanthro[2,3-d]thiophene			0.11	0.038			5.0	0.70	Fertuck et al. (2001)
RUC	4-Hydroxychalcone	20426-12-4						0.0028	-2.430	Fang et al. (2001)
RUC	4'-Hydroxychalcone	2657-25-2						0.0037	-2.430	Fang et al. (2001)
RUC	4'-Hydroxychalcone (*cis*- and *trans*-)	38239-52-0						0.0037	-2.550	Fang et al. (2001)
GST-hERαdef	2-Hydroxychrysene	65945-06-4		0.095	0.044			5.80	0.76	Fertuck et al. (2001)
hERβ	2-Hydroxychrysene	65945-06-4		0.042	0.014			13.33	1.12	Fertuck et al. (2001)
RUC	4'-Hydroxy-2,3-diphenylindenone-1							0.45	-0.347	Anstead et al. (1989)
RUC	6'-Hydroxy-2,3-diphenylindenone-1							59	1.771	Anstead et al. (1989)
MCF-7 cytosol	11α-Hydroxyestradiol	1464-61-5						3.1	0.491	Palomino et al. (1994)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (µM)**	SD of IC_{50}**	HDT (µM)	Ki (µM)**	RBA***	log RBA***	Reference
RBC	11β-Hydroxyestradiol	5444-22-4						4	0.602	Korenman (1969)
MCF-7 cytosol	11β-Hydroxyestradiol	5444-22-4						16.8	1.225	Palomino et al. (1994)
RUC	11β-Hydroxyestradiol	5444-22-4						7	0.845	Qian and Abul-Hajj (1990)
MCF-7 cells	11β-Hydroxyestradiol	5444-22-4						4	0.602	Stoessel and Leclerq (1986)
MCF-7 cytosol	11β-Hydroxyestradiol	5444-22-4						1	0.000	Stoessel and Leclerq (1986)
MCF-7 cells	14β-Hydroxyestradiol	60183-66-6						3.5	0.544	Stoessel and Leclerq (1986)
MCF-7 cytosol	14β-Hydroxyestradiol	60183-66-6						10	1.000	Stoessel and Leclerq (1986)
hERα	2-Hydroxyestradiol	362-05-0					0.0025	7	0.845	Kuiper et al. (1997)
rERβ	2-Hydroxyestradiol	362-05-0					0.0013	11	1.040	Kuiper et al. (1997)
hERα	4-Hydroxyestradiol	5976-61-4					0.001	13	1.114	Kuiper et al. (1997)
rERβ	4-Hydroxyestradiol	5976-61-4					0.0019	7	0.845	Kuiper et al. (1997)
MCF-7 cytosol	2-Hydroxyestratrien-17β-ol	2259-89-4						18	1.255	Brooks et al. (1987)
MCF-7 cytosol	4-Hydroxyestratrien-17β-ol	17592-89-1						0.8	-0.097	Brooks et al. (1987)
RUC	3-Hydroxyestra-1,3,5(10)-trien-16-one	3601-97-6		0.175	0.005			0.51	-0.290	Blair et al. (2000)
hERα	2-Hydroxyestrone	362-06-1						2	0.301	Kuiper et al. (1998) [method a]
hERβ	2-Hydroxyestrone	362-06-1						0.2	-0.699	Kuiper et al. (1998) [method a]
hERα-FP	2-Hydroxyethyl methacrylate	868-77-9	95			5000				Hashimoto et al. (2000)
RUC	3'-Hydroxyflavanone	92496-65-6						0.0017	-2.770	Fang et al. (2001)
RUC	4'-Hydroxyflavanone	135413-27-3						0.0023	-2.640	Fang et al. (2001)
RUC	6-Hydroxyflavanone	4250-77-5						0.0009	-3.050	Fang et al. (2001)
RUC	7-Hydroxyflavanone	6515-36-2						0.00019	-3.720	Fang et al. (2001)
RUC	6-Hydroxyflavone	6665-83-4						0.0004	-3.398	Fang et al. (2001)
RUC	7-Hydroxyflavone	6665-86-7				100				Fang et al. (2001)
RUC	Hydroxyflutamide	52806-53-8				*1000*				Laws et al. (1996)
RUC	Hydroxyflutamide	52806-53-8					1000	0.0003	-3.523	Waller et al. (1996)
RUC	2-Hydroxy-4-methoxybenzophenone	131-57-7	98			100				Blair et al. (2000)
RUC	6-Hydroxy-2'-methoxyflavone	61546-59-6				100				Fang et al. (2001)
GST-hERαdef	2-Hydroxy-5-methylchrysene			0.028	0.012			19.60	1.29	Fertuck et al. (2001)
hERβ	2-Hydroxy-5-methylchrysene			0.029	0.005			19.30	1.29	Fertuck et al. (2001)
GST-hERαdef	8-Hydroxy-5-methylchrysene			0.18	0.026			3.10	0.49	Fertuck et al. (2001)
hERβ	8-Hydroxy-5-methylchrysene			0.18	0.032			3.10	0.49	Fertuck et al. (2001)
RUC	16β-Hydroxy-16-methyl-17β-estradiol 3-methyl ether	3434-79-5		2.7	0.2			0.033	-1.480	Blair et al. (2000)
hERα	4-Hydroxytamoxifen	68047-06-3					0.0001	178	2.250	Kuiper et al. (1997)
hERα	4-Hydroxytamoxifen	68047-06-3						257	2.410	Kuiper et al. (1998) [method a]
hERα	4-Hydroxytamoxifen	68047-06-3					0.00022	149	2.173	Sun et al. (1999)
hERα-FP	4-Hydroxytamoxifen	68047-06-3	> 93	*0.01*				*400*	2.602	Saito et al. (2000)
hERβ	4-Hydroxytamoxifen	68047-06-3						232	2.365	Kuiper et al. (1998) [method a]
hERβ	4-Hydroxytamoxifen	68047-06-3					0.0015	62	1.792	Sun et al. (1999)
MUC	4-Hydroxytamoxifen	68047-06-3	> 99	*0.0125*				*14.4*	*1.150*	Shelby et al. (1996)
rERβ	4-Hydroxytamoxifen	68047-06-3					0.00004	339	2.530	Kuiper et al. (1997)
GST-aERdef	4-Hydroxytamoxifen	68047-06-3		0.0013	0.0001			243	2.386	Matthews et al. (2000)
GST-cERdef	4-Hydroxytamoxifen	68047-06-3		0.0019	0.0003			168	2.225	Matthews et al. (2000)
GST-hERαdef	4-Hydroxytamoxifen	68047-06-3		0.0019	0.0001			155	2.190	Matthews et al. (2000)
GST-mERαdef	4-Hydroxytamoxifen	68047-06-3		0.0012	0.0004			212	2.326	Matthews et al. (2000)
GST-rtERdef	4-Hydroxytamoxifen	68047-06-3		0.0012	0.0009			272	2.435	Matthews et al. (2000)
hERα-FP	4-Hydroxytamoxifen	68047-06-3		0.096	0.0008			14	1.146	Nikov et al. (2001)
hERα-FP	4-Hydroxytamoxifen	68047-06-3		0.026				21.5	1.332	Parker et al. (2000)
RUC	4-Hydroxytamoxifen	68047-06-3		0.000513	0.000112			175.244	2.240	Blair et al. (2000)
MCF-7 cells	4-Hydroxytamoxifen	68047-06-3						2.9	0.462	Stoessel and Leclerq (1986)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (μM)**	SD of IC_{50}**	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
MCF-7 cytosol	4-Hydroxytamoxifen	68047-06-3						100	2.000	Stoessel and Leclerq (1986)
RUC	6-Hydroxytetralin	1125-78-6				500				Elsby et al. (2000)
GST-aERdef	ICI 164384	98007-99-9		0.011	0.002			28	1.447	Matthews et al. (2000)
GST-cERdef	ICI 164384	98007-99-9		0.0052	0.001			62	1.792	Matthews et al. (2000)
GST-hERαdef	ICI 164384	98007-99-9		0.007	0.0003			42	1.623	Matthews et al. (2000)
GST-mERαdef	ICI 164384	98007-99-9		0.0059	0.0003			45	1.653	Matthews et al. (2000)
GST-rtERdef	ICI 164384	98007-99-9		0.001	0.0007			327	2.515	Matthews et al. (2000)
hERα	ICI 164384	98007-99-9					0.0002	85	1.929	Kuiper et al. (1997)
hERα	ICI 164384	98007-99-9		0.00059				158.0	2.200	Kuiper et al. (1998) [method b]
hERβ	ICI 164384	98007-99-9		0.00114				93.0	1.970	Kuiper et al. (1998) [method b]
rERβ	ICI 164384	98007-99-9					0.00008	166	2.220	Kuiper et al. (1997)
RUC	ICI 164384	98007-99-9		0.0062	0.0013			14.5	1.160	Blair et al. (2000)
hERα	ICI 182780	129453-61-8					0.001	32	1.505	Sun et al. (1999)
hERβ	ICI 182780	129453-61-8					0.0036	25	1.398	Sun et al. (1999)
RBC	ICI 182780	129453-61-8	99.3	0.000004				500	2.699	Andersen et al. (1999)
RUC	ICI 182780	129453-61-8		0.0024	0.0011			37.46	1.570	Blair et al. (2000)
RUC	ICI 182780	129453-61-8					0.00059979	433	2.636	Waller et al. (1996)
hERα-FP	Imiprothrin	72963-72-5	> 93			10				Saito et al. (2000)
MUC	Indanestrol	71855-45-3		0.05	0.005			2	0.301	Korach et al. (1979)
MUC	Indanestrol	71855-45-3		6	5			2.67	0.427	Korach et al. (1985)
MUC	Indanyldiethylstilbestrol			0.002	0.0004			80	1.900	Korach et al. (1978)
MUC	Indenestrol A	24643-97-8		0.0007	0.0001			143	2.155	Korach et al. (1979)
MUC	Indenestrol A	24643-97-8		0.07	0.1			229	2.360	Korach et al. (1985)
MUC	(*R*)-Indenestrol A	115217-03-3						13	1.110	Korach et al. (1989)
MUC	(*rac*)-Indenestrol A	115217-02-2						143	2.155	Korach et al. (1989)
MUC	(*S*)-Indenestrol A	115217-04-4						285	2.460	Korach et al. (1989)
MUC	Indenestrol B	38028-27-2		0.0007	0.0002			143	2.155	Korach et al. (1979)
MUC	Indenestrol B	38028-27-2		0.7	0.1			229	2.360	Korach et al. (1985)
MUC	(*R*)-Indenestrol B	115217-06-6						100	2.000	Korach et al. (1989)
MUC	(rac) Indenestrol B	133830-97-4						145	2.160	Korach et al. (1989)
MUC	(*S*)-Indenestrol B							143	2.160	Korach et al. (1989)
MCF-7 cells	Indeno[1,2,3-*cd*]pyrene	193-39-5						20	1.301	Arcaro et al. (1999)
RUC	Indole[3,2-β]carbazole		> 98	23				0.00083	-3.081	Liu et al. (1994)
MCF-7 cytosol	16α-Iodoestradiol	71765-94-1		*0.006*				100	2.000	Miodini et al. (1999)
MCF-7 cytosol	(*E*)-17α-Iodovinylestradiol	82123-96-4					0.00104	17	1.230	Rijks et al. (1996)
RUC	(*E*)-17α-Iodovinylestradiol	82123-96-4					0.0022	7	0.845	Rijks et al. (1996)
MCF-7 cytosol	(*Z*)-17α-iodovinylestradiol	177159-09-0					0.00039	51	1.708	Rijks et al. (1996)
RUC	(*Z*)-17α-iodovinylestradiol	177159-09-0					0.00025	63	1.799	Rijks et al. (1996)
hERα	Ipriflavone	35212-22-7				10				Kuiper et al. (1998) [method a]
hERβ	Ipriflavone	35212-22-7				10				Kuiper et al. (1998) [method a]
RUC	Isoeugenol	97-54-1	98			100				Blair et al. (2000)
hERα	Kaempferol	520-18-3						0.1	-1.000	Kuiper et al. (1998) [method a]
hERβ	Kaempferol	520-18-3						3	0.477	Kuiper et al. (1998) [method a]
hERβ	Kaempferol	520-18-3		0.054				2	0.301	Kuiper et al. (1998) [method b]
RUC	Kaempferol	520-18-3						0.025	-1.600	Fang et al. (2001)
GST-aERdef	Kepone	143-50-0		27	7			0.011	-1.959	Matthews et al. (2000)
GST-cERdef	Kepone	143-50-0		30	1			0.011	-1.959	Matthews et al. (2000)
GST-hERαdef	Kepone	143-50-0		42	18			0.0069	-2.161	Matthews et al. (2000)
GST-mERαdef	Kepone	143-50-0		64	3			0.0035	-2.456	Matthews et al. (2000)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (µM)**	SD of IC_{50}**	HDT (µM)	Ki (µM)**	RBA***	log RBA***	Reference
GST-rtERdef	Kepone	143-50-0		6.2	0.4			0.054	-1.268	Matthews et al. (2000)
hERα	Kepone	143-50-0						0.06	-1.222	Kuiper et al. (1998) [method a]
hERα-FP	Kepone	143-50-0	87.5	5.7				0.2	-0.699	Bolger et al. (1998)
hERβ	Kepone	143-50-0						0.1	-1.000	Kuiper et al. (1998) [method a]
MUC	Kepone	143-50-0	98			5				Shelby et al. (1996)
RUC	Kepone	143-50-0		7.0	1.00			0.013	-1.890	Blair et al. (2000)
RUC	Kepone	143-50-0		4.005			1.5	0.03	-0.570	Laws et al. (2000)
RUC	Kepone	143-50-0					1.40	0.1862	-0.730	Waller et al. (1996)
MCF-7 cytosol	11-Keto-9β-estradiol					3				Palomino et al. 1994
hERα	16-Ketoestradiol	566-75-6						1.3	0.000	Kuiper et al. (1998) [method a]
hERβ	16-Ketoestradiol	566-75-6						0.9	-0.046	Kuiper et al. (1998) [method a]
MCF-7 cytosol	16-Ketoestradiol	566-75-6						0.9	-0.046	Palomino et al. (1994)
RBC	16-Ketoestradiol	566-75-6						14	1.146	Korenman (1969)
MCF-7 cells	6-Ketoestradiol	571-92-6						15	1.176	Stoessel and Leclerq (1986)
MCF-7 cytosol	6-Ketoestradiol	571-92-6						20	1.301	Stoessel and Leclerq (1986)
RUC	Lindane	58-89-9	99			100				Blair et al. (2000)
RUC	Lindane	58-89-9					1000	0.0003	-3.523	Waller et al. (1996)
RUC	Melatonin	73-31-4	97			100				Blair et al. (2000)
RBC	MER-25	67-98-1						0.00096	-3.018	Korenman (1970)
RUC	Mestilbol	18839-90-2		0.0044	0.0005			20.43	1.310	Blair et al. (2000)
RBC	Mestranol	72-33-3						8	0.903	Korenman (1969)
RUC	Mestranol	72-33-3	98	2.5				0.25	-0.541	Allen et al. (1980)
RUC	Mestranol	72-33-3		0.0397	0.0065			2.26	0.350	Blair et al. (2000)
GST-aERdef	*p,p'* - Methoxychlor	72-43-5				*100*				Matthews et al. (2000)
GST-cERdef	*p,p'* - Methoxychlor	72-43-5				*100*				Matthews et al. (2000)
GST-hERαdef	*p,p'* - Methoxychlor	72-43-5				*100*				Matthews et al. (2000)
GST-mERαdef	*p,p'* - Methoxychlor	72-43-5				*100*				Matthews et al. (2000)
GST-rtERdef	*p,p'* - Methoxychlor	72-43-5		3.5	0.4			0.95	-0.022	Matthews et al. (2000)
hERα	*p,p'* - Methoxychlor	72-43-5					1.77	0.01	-2.000	Kuiper et al. (1997)
hERα	*p,p'* - Methoxychlor	72-43-5				10				Kuiper et al. (1998) [method a]
hERα-FP	*p,p'* - Methoxychlor	72-43-5	98	*135*				*0.0096*	-2.018	Bolger et al. (1998)
hERβ	*p,p'* - Methoxychlor	72-43-5				10				Kuiper et al. (1998) [method a]
MCF-7 cytosol	*p,p'* - Methoxychlor	72-43-5				1000				Dodge et al. (1996)
MUC	*p,p'* - Methoxychlor	72-43-5	98			5				Shelby et al. (1996)
RBC	*p,p'* - Methoxychlor	72-43-5	98.4	6.5				0.0031	-2.509	Andersen et al. (1999)
rERβ	*p,p'* - Methoxychlor	72-43-5					0.09	0.13	-0.886	Kuiper et al. (1997)
RUC	*p,p'* - Methoxychlor	72-43-5	95	144	66			0.001	-3.200	Blair et al. (2000)
RUC	*p,p'* - Methoxychlor	72-43-5	99			100				Blair et al. (2000)
RUC	*p,p'* - Methoxychlor	72-43-5	95	174			65	0.00062	-3.210	Laws et al. (2000)
RUC	*p,p'* - Methoxychlor	72-43-5				*100*				Nelson (1974)
RUC	*p,p'* - Methoxychlor	72-43-5					69.02	0.0038	-2.420	Waller et al. (1996)
RUC	Methoxychlor olefin	2132-70-9	95			100				Blair et al. (2000)
RUC	3-Methoxyestriol	1474-53-9		4	0			0.022	-1.650	Blair et al. (2000)
MCF-7 cytosol	*E* -11β-methoxy-17α-iodovinylestradiol	90857-55-9					0.00104	17	1.230	Rijks et al. (1996)
RUC	*E* -11β-methoxy-17α-iodovinylestradiol	90857-55-9					0.0014	11	1.041	Rijks et al. (1996)
MCF-7 cytosol	(*Z*)-11β-methoxy-17α-iodovinylestradiol	177159-11-4					0.00059	31	1.491	Rijks et al. (1996)
RUC	(*Z*)-11β-methoxy-17α-iodovinylestradiol	177159-11-4					0.0004	41	1.613	Rijks et al. (1996)
RUC	3-Methoxy-10-methyl-11-phenyldibenzo[*b,f*]thiepin (16β)	85807-06-1						0.005	-2.301	Acton et al. (1983)
RUC	Methoxytamoxifen			0.85				0.74	0.735	Allen et al. (1980)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (μM)**	SD of IC_{50}**	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
RUC	7α-Methyl-14-dehydroestradiol-17β	88598-62-3						74	1.869	Gabbard and Segaloff (1983)
RUC	9α-Methyl-14-dehydroestradiol-17β	88598-63-4						41	1.613	Gabbard and Segaloff (1983)
RUC	7α-Methyl-14-dehydroestradiol-17β 3-methyl ether	35644-59-8						3.1	0.491	Gabbard and Segaloff (1983)
RUC	9α-Methyl-14-dehydroestradiol-17β 3-methyl ether	88598-64-5						0.1	-1.000	Gabbard and Segaloff (1983)
RUC	11β-Methyl-14-dehydroestradiol-17β 3-methyl ether	88598-65-6						1.2	0.079	Gabbard and Segaloff (1983)
RUC	7α-Methyl-14-dehydroestrone	88958-66-7						52	1.716	Gabbard and Segaloff (1983)
RUC	9α-Methyl-14-dehydroestrone	88598-67-8						6	0.778	Gabbard and Segaloff (1983)
RUC	7α-Methyl-14-dehydroestrone 3-methyl ether	35644-57-6				1				Gabbard and Segaloff (1983)
RUC	9α-Methyl-14-dehydroestrone 3-methyl ether					1				Gabbard and Segaloff (1983)
RUC	11β-Methyl-14-dehydroestrone 3-methyl ether	88598-69-0				1				Gabbard and Segaloff (1983)
RUC	4,4'-Methylenebis(N,N-dimethylaniline)	101-61-1	98			1000				Blair et al. (2000)
RUC	4,4'-Methylenedianiline	101-77-9	97			233				Blair et al. (2000)
RUC	7α Methylestradiol-17β	10448-97-2						104	2.017	Gabbard and Segaloff (1983)
RUC	9α Methylestradiol-17β	66463-44-3						35	1.544	Gabbard and Segaloff (1983)
MCF-7 cells	11β-Methylestradiol-17β	23637-93-6						100	2.000	Stoessel and Leclerq (1986)
MCF-7 cytosol	11β-Methylestradiol-17β	23637-93-6						100	2.000	Stoessel and Leclerq (1986)
RUC	11β-Methylestradiol-17β	23637-93-6						124	2.093	Gabbard and Segaloff (1983)
RUC	7α Methylestradiol-17β 3-methyl ether	15506-01-1						5.3	0.724	Gabbard and Segaloff (1983)
RUC	9α-Methylestradiol-17β 3-methyl ether	51242-32-1				1				Gabbard and Segaloff (1983)
RUC	11β-Methylestradiol-17β 3-methyl ether	18046-75-8						5.1	0.708	Gabbard and Segaloff (1983)
RUC	7α-Methylestrone	10448-96-1						68	1.833	Gabbard and Segaloff (1983)
RUC	9α-Methylestrone	71563-77-4						5	0.699	Gabbard and Segaloff (1983)
RUC	11β-Methylestrone	13667-06-6						47	1.672	Gabbard and Segaloff (1983)
RUC	7α-Methylestrone 3-methyl ether	10449-00-0				1				Gabbard and Segaloff (1983)
RUC	9α-Methylestrone 3-methyl ether	31266-41-8				1				Gabbard and Segaloff (1983)
RUC	11β-Methylestrone 3-methyl ether	13667-04-4				1				Gabbard and Segaloff (1983)
RUC	1-Methyl-3-ethyl-6,4'-dihydroxy-2-phenylindene							81	1.908	Anstead et al. (1989)
RUC	1-Methyl-6-hydroxy-2,3-diphenylindene							12	1.079	Anstead et al. (1989)
hERα-FP	Methyl methacrylate	80-62-6	> 98			5000				Hashimoto et al. (2000)
RUC	Methyl paraben	99-76-3	99	245	65			0.0004	-3.440	Blair et al. (2000)
RUC	2-(2-Methylphenyl)-3-phenyl-6-hydroxyindene							100	2.000	Anstead et al. (1990)
RUC	Methyltamoxifen	73617-95-5		0.0075				0.3	-0.900	Allen et al. (1980)
RUC	*o,p'* -Methyoxychlor	30667-99-3		*9*				*0.01*	-2.000	Nelson (1974)
RUC	Metolachlor	51218-45-2	98.7			100				Blair et al. (2000)
RUC	Mirex	2385-85-5	99			100				Blair et al. (2000)
RUC	Mono-*m* -acetoxy-1,1,2-triphenylbut-1-ene	82333-69-5						1	0.000	Jordan et al. (1986)
RUC	Monohydroxymethoxychlor	28463-03-8	98	0.69	0.01			0.13	-0.890	Blair et al. (2000)
RUC	Monohydroxymethoxychlor olefin	75938-34-0	98	0.39	0.08			0.23	-0.640	Blair et al. (2000)
RUC	Monohydroxytamoxifen	68392-35-8		0.012				52.1	1.800	Allen et al. (1980)
RUC	Morin	480-16-0						0.00045	-3.350	Fang et al. (2001)
hERα	Moxestrol	34816-55-2					0.0005	43	1.633	Kuiper et al. (1997)
MUC	Moxestrol	34816-55-2						4.25	0.628	Korach (1979)
rERβ	Moxestrol	34816-55-2					0.0026	5	0.699	Kuiper et al. (1997)
RUC	Moxestrol	34816-55-2		0.0065	0.0014			13.83	1.140	Blair et al. (2000)
RUC	Myricetin	529-44-2						0.0018	-2.740	Fang et al. (2001)
hERα	Nafoxidine	1845-11-0					0.0003	44	1.643	Kuiper et al. (1997)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (μM)**	SD of IC_{50}**	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
rERβ	Nafoxidine	1845-11-0					0.0008	16	1.204	Kuiper et al. (1997)
RUC	Nafoxidine	1845-11-0		0.125	0.055			0.72	-0.140	Blair et al. (2000)
MCF-7 cells	Nafoxidine	1845-11-0						0.1	-1.000	Stoessel and Leclerq (1986)
MCF-7 cytosol	Nafoxidine	1845-11-0						5	0.699	Stoessel and Leclerq (1986)
GST-aERdef	Naringenin	480-41-1		4.7	0.8			0.065	-1.187	Matthews et al. (2000)
GST-cERdef	Naringenin	480-41-1		39	4			0.0082	-2.086	Matthews et al. (2000)
GST-hERαdef	Naringenin	480-41-1				*100*				Matthews et al. (2000)
GST-mERαdef	Naringenin	480-41-1				*100*				Matthews et al. (2000)
GST-rtERdef	Naringenin	480-41-1		8.7	1.3			0.039	-1.409	Matthews et al. (2000)
hERα	Naringenin	480-41-1						0.01	-2.000	Kuiper et al. (1998) [method a]
hERβ	Naringenin	480-41-1						0.11	-0.959	Kuiper et al. (1998) [method a]
hERβ	Naringenin	480-41-1		0.59				0.2	-0.699	Kuiper et al. (1998) [method b]
RUC	Naringenin	480-41-1						0.0075	-2.120	Fang et al. (2001)
RUC	Naringin	10236-47-2				100				Fang et al. (2001)
RUC	Nerolidol	7212-44-4	97.7			1000				Blair et al. (2000)
MCF-7 cytosol	4-Nitroesratrien-3-ol-17-one	5976-74-9						6	0.778	Brooks et al. (1987)
MCF-7 cytosol	2-Nitroestratriene-3,17β-diol	6298-51-7						1	0.000	Brooks et al. (1987)
MCF-7 cytosol	4-Nitroestratriene-3,17β-diol	6936-94-3						13	1.114	Brooks et al. (1987)
MCF-7 cytosol	2-Nitroestratrien-3-ol-17-one	5976-73-8						0.1	-1.000	Brooks et al. (1987)
RBC	Nitromifene	10448-84-7						0.12	-0.924	Korenman (1970)
hERα	*cis* -Nonachlor	5103-73-1				50				Klotz et al. (1996)
hERα	*trans* -Nonachlor	39765-80-5				50				Klotz et al. (1996)
RUC	Nonylbenzene	1081-77-2				*500*				Elsby et al. (2000)
hERα	*n* -Nonylphenol	25154-52-3						0.05	-1.301	Kuiper et al. (1998) [method a]
hERα	*n* -Nonylphenol	25154-52-3		500				0.001	-3.00	Morito et al. (2001)
hERβ	*n* -Nonylphenol	25154-52-3						0.09	-1.046	Kuiper et al. (1998) [method a]
hERβ	*n* -Nonylphenol	25154-52-3		8				0.063	-1.20	Morito et al. (2001)
MUC	*n* -Nonylphenol	25154-52-3	99.5			5				Shelby et al. (1996)
RBC	*n* -Nonylphenol	25154-52-3	99.9	1.8				0.0011	-2.959	Andersen et al. (1999)
hERα-FP	*p* -Nonylphenol	104-40-5	85	3.9				0.3	-0.523	Bolger et al. (1998)
hERα-FP	*p* -Nonylphenol	104-40-5	> 93	*7*				*0.5*	-0.301	Saito et al. (2000)
MCF-7 cells	*p* -Nonylphenol	104-40-5	Technical grade					0.026	-1.585	Nagel et al. (1997)
MCF-7 cytosol	*p* -Nonylphenol	104-40-5		7.2	3			0.021	-1.678	Soto et al. (1995)
RUC	*p* -Nonylphenol	104-40-5	85	4.73	0.93			0.019	-1.720	Blair et al. (2000)
RUC	*p* -Nonylphenol	104-40-5	Technical grade	3.05	0.15			0.029	-1.530	Blair et al. (2000)
RUC	*p* -Nonylphenol	104-40-5	85	2.9	0.8			0.031	-1.510	Blair et al. (2000)
RUC	*p* -Nonylphenol	104-40-5	Technical grade	2.6	0.3			0.035	-1.460	Blair et al. (2000)
RUC	*p* -Nonylphenol	104-40-5	95.6	2.4	0.3			0.037	-1.430	Blair et al. (2000)
RUC	*p* -Nonylphenol	104-40-5	98	28	10			0.0032	-2.490	Blair et al. (2000)
RUC	*p* -Nonylphenol	104-40-5		*3*				*0.17*	-0.770	Elsby et al. (2000)
RUC	*p* -Nonylphenol	104-40-5	85	0.1794			0.67	0.158	-0.800	Laws et al. (2000)
RUC	*p* -Nonylphenol	104-40-5	95	8				0.0025	-2.602	Routledge et al. (1998)
RUC	*p* -Nonylphenol	104-40-5					0.83	0.31	-0.504	Waller et al. (1996)
RBC	Nonylphenol dodecylethoxylate					100				Andersen et al. (1999)
RUC	Nordihydroguariaretic acid	500-38-9	97	2.9	1.6			0.031	-1.510	Blair et al. (2000)
hERα	Norethindrone	68-22-4					0.15	0.07	-1.155	Kuiper et al. (1997)
rERβ	Norethindrone	68-22-4					1.08	0.01	-2.000	Kuiper et al. (1997)
hERα	Norethynodrel	68-23-5					0.014	0.7	-0.155	Kuiper et al. (1997)
rERβ	Norethynodrel	68-23-5					0.053	0.22	-0.658	Kuiper et al. (1997)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (µM)**	SD of IC_{50}**	HDT (µM)	Ki (µM)**	RBA***	log RBA***	Reference
RUC	Norethynodrel	68-23-5		0.44	0.04			0.20	-0.690	Blair et al. (2000)
RUC	Norethynodrel	68-23-5	99	0.4	0.010			0.23	-0.650	Blair et al. (2000)
hERα	19-Nortestosterone	434-22-0					0.77	0.01	-2.000	Kuiper et al. (1997)
MUC	19-Nortestosterone	434-22-0						0.1	0.950	Korach (1979)
rERβ	19-Nortestosterone	434-22-0					0.053	0.23	-0.638	Kuiper et al. (1997)
GST-aERdef	2,2'3,3',4,4',5,5'-Octachlorobiphenyl	35694-08-7				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2'3,3',4,4',5,5'-Octachlorobiphenyl	35694-08-7				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2'3,3',4,4',5,5'-Octachlorobiphenyl	35694-08-7				10				Matthews and Zacharewski (2000)
RUC	1,8-Octanediol	629-41-4	98			100				Blair et al. (2000)
hERβ	4-*n* -Octylphenol	1806-26-4						0.07	-1.155	Kuiper et al. (1998) [method a]
MCF-7 cells	4-*n* -Octylphenol	1806-26-4	Technical grade					0.072	-1.143	Nagel et al. (1997)
MCF-7 cytosol	4-*n* -Octylphenol	1806-26-4		*0.9*				1	0.000	Dodge et al. (1996)
RBC	4-*n* -Octylphenol	1806-26-4	99.4			100				Andersen et al. (1999)
hERα	4-*n* -Octylphenol	1806-26-4						0.02	-1.699	Kuiper et al. (1998) [method a]
RUC	4-*n* -Octylphenol	1806-26-4	99	19.5	1.5			0.005	-2.340	Blair et al. (2000)
GST-aERdef	4-*tert* -Octylphenol	140-66-9		3.9	1.6			0.099	-1.004	Matthews et al. (2000)
GST-cERdef	4-*tert* -Octylphenol	140-66-9		0.56	0.01			0.57	-0.244	Matthews et al. (2000)
GST-hERαdef	4-*tert* -Octylphenol	140-66-9		2.4	0.7			0.12	-0.921	Matthews et al. (2000)
GST-mERαdef	4-*tert* -Octylphenol	140-66-9		1.6	0.1			0.17	-0.770	Matthews et al. (2000)
GST-rtERdef	4-*tert* -Octylphenol	140-66-9		0.11	0.02			3.2	0.505	Matthews et al. (2000)
hERα	4-*tert* -Octylphenol	140-66-9						0.01	-2.000	Kuiper et al. (1998) [method a]
hERα-FP	4-*tert* -Octylphenol	140-66-9	97	7.5				0.2	-0.699	Bolger et al. (1998)
hERβ	4-*tert* -Octylphenol	140-66-9						0.03	-1.523	Kuiper et al. (1998) [method a]
RUC	4-*tert* -Octylphenol	140-66-9					1.32	0.197	-0.706	Waller et al. (1996)
RUC	4-*tert* -Octylphenol	140-66-9	97	6.0	1.10			0.015	-1.820	Blair et al. (2000)
RUC	4-*tert* -Octylphenol	140-66-9	97	0.2085			0.78	0.51	-0.291	Laws et al. (2000)
GST-aERdef	2,2',3,3',6-Pentachlorobiphenyl	52663-60-2				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3,3',6-Pentachlorobiphenyl	52663-60-2				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,3',6-Pentachlorobiphenyl	52663-60-2				10				Matthews and Zacharewski (2000)
GST-aERdef	2,2',3,4,5'-Pentachlorobiphenyl	38380-02-8				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3,4,5'-Pentachlorobiphenyl	38380-02-8				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,4,5'-Pentachlorobiphenyl	38380-02-8				10				Matthews and Zacharewski (2000)
GST-aERdef	2,2',3,4',6-Pentachlorobiphenyl	68194-05-8				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3,4',6-Pentachlorobiphenyl	68194-05-8				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,4',6-Pentachlorobiphenyl	68194-05-8		10				0.031	-1.509	Matthews and Zacharewski (2000)
GST-aERdef	2,2',3,5',6-Pentachlorobiphenyl	38379-99-6				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3,5',6-Pentachlorobiphenyl	38379-99-6				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,5',6-Pentachlorobiphenyl	38379-99-6				10				Matthews and Zacharewski (2000)
GST-aERdef	2,2',4,4',5-Pentachlorobiphenyl	38380-01-7				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',4,4',5-Pentachlorobiphenyl	38380-01-7				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',4,4',5-Pentachlorobiphenyl	38380-01-7				10				Matthews and Zacharewski (2000)
GST-aERdef	2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2				10				Matthews and Zacharewski (2000)
GST-aERdef	2,2',4,6,6'-Pentachlorobiphenyl	56558-16-8		10				0.025	-1.602	Matthews and Zacharewski (2000)
GST-hERαdef	2,2',4,6,6'-Pentachlorobiphenyl	56558-16-8		10				0.024	-1.620	Matthews and Zacharewski (2000)
GST-rtERdef	2,2',4,6,6'-Pentachlorobiphenyl	56558-16-8		1.3	0.6			0.24	-0.623	Matthews and Zacharewski (2000)
MUC	2,2',4,6,6'-Pentachlorobiphenyl	56558-16-8	> 98	1.7				0.88	-0.056	Fielden et al. (1997)
GST-aERdef	2,3,3',5,6,-Pentachlorobiphenyl	74472-36-9				10				Matthews and Zacharewski (2000)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (µM)**	SD of IC_{50}**	HDT (µM)	Ki (µM)**	RBA***	log RBA***	Reference
GST-hERαdef	2,3,3',5,6,-Pentachlorobiphenyl	74472-36-9				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,3,3',5,6,-Pentachlorobiphenyl	74472-36-9				10				Matthews and Zacharewski (2000)
GST-aERdef	2,3,4,4',6,-Pentachlorobiphenyl	74472-38-1				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,3,4,4',6,-Pentachlorobiphenyl	74472-38-1				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,3,4,4',6,-Pentachlorobiphenyl	74472-38-1		10				0.031	-1.509	Matthews and Zacharewski (2000)
GST-aERdef	3,3',4,4',5,-Pentachlorobiphenyl	57465-28-8				10				Matthews and Zacharewski (2000)
GST-hERαdef	3,3',4,4',5,-Pentachlorobiphenyl	57465-28-8				10				Matthews and Zacharewski (2000)
GST-rtERdef	3,3',4,4',5,-Pentachlorobiphenyl	57465-28-8				10				Matthews and Zacharewski (2000)
hERα	2,2',3',4',5'-Pentachloro-4-biphenylol	150304-12-4	> 98					0.1	-1.000	Kuiper et al. (1998) [method a]
hERβ	2,2',3',4',5'-Pentachloro-4-biphenylol	150304-12-4	> 98					0.13	-0.886	Kuiper et al. (1998) [method a]
MUC	2,2',3',4',5'-Pentachloro-4-biphenylol	150304-12-4	> 98	6.3				0.033	-1.480	Connor et al. (1997)
RUC	2,2',3',4',5'-Pentachloro-4-biphenylol	150304-12-4	> 98	40				0.036	-1.440	Connor et al. (1997)
hERα	2,2',3',4',6'-Pentachloro-4-biphenylol	150304-10-2	> 98					0.3	-0.523	Kuiper et al. (1998) [method a]
hERβ	2,2',3',4',6'-Pentachloro-4-biphenylol	150304-10-2	> 98					0.2	-0.699	Kuiper et al. (1998) [method a]
MUC	2,2',3',4',6'-Pentachloro-4-biphenylol	150304-10-2	> 98	4.8				0.044	-1.360	Connor et al. (1997)
RUC	2,2',3',4',6'-Pentachloro-4-biphenylol	150304-10-2	> 98	12				0.12	-0.920	Connor et al. (1997)
hERα	2,2',3',5',6'-Pentachloro-4-biphenylol	150304-11-3	> 98					0.09	-1.046	Kuiper et al. (1998) [method a]
hERβ	2,2',3',5',6'-Pentachloro-4-biphenylol	150304-11-3	> 98					0.03	-1.523	Kuiper et al. (1998) [method a]
MUC	2,2',3',5',6'-Pentachloro-4-biphenylol	150304-11-3	> 98	16				0.013	-1.890	Connor et al. (1997)
RUC	2,2',3',5',6'-Pentachloro-4-biphenylol	150304-11-3	> 98	10				0.14	-0.850	Connor et al. (1997)
MUC	2,2',4,6,6'-Pentachloro-4-biphenylol		> 98	0.07				21.43	1.331	Fielden et al. (1997)
MCF-7 cytosol	2',3,3',4,4'-Pentachloro-2-biphenylol	150975-80-7	> 95	5.7	0.2			0.004	-2.398	Kramer et al. (1997)
hERα	2',3,3',4',5-Pentachloro-4-biphenylol	192190-09-3	> 98					0.01	-2.000	Kuiper et al. (1998) [method a]
hERα	2',3,3',4',5'-Pentachloro-4-biphenylol	149589-55-9	> 98					0.11	-0.959	Kuiper et al. (1998) [method a]
hERβ	2',3,3',4',5-Pentachloro-4-biphenylol	192190-09-3	> 98			10				Kuiper et al. (1998) [method a]
hERβ	2',3,3',4',5'-Pentachloro-4-biphenylol	149589-55-9	> 98					0.11	-0.959	Kuiper et al. (1998) [method a]
MUC	2',3,3',4',5'-Pentachloro-4-biphenylol	149589-55-9	> 98	2.9				0.072	-1.140	Connor et al. (1997)
RUC	2',3,3',4',5'-Pentachloro-4-biphenylol	149589-55-9	> 98	17				0.082	-1.090	Connor et al. (1997)
hERα	2,3,3',4',5-Pentachloro-4-biphenylol	152969-11-4	> 98					0.03	-1.523	Kuiper et al. (1998) [method a]
hERβ	2,3,3',4',5-Pentachloro-4-biphenylol	152969-11-4	> 98					0.11	-1.699	Kuiper et al. (1998) [method a]
MCF-7 cytosol	2,3,3',4',5-Pentachloro-4-biphenylol	152969-11-4	> 95	3.3	0.2			1	0.000	Kramer et al. (1997)
MCF-7 cytosol	2',3,3',4',5-Pentachloro-4-biphenylol	192190-09-3	> 95	4.2				0.1	-1.000	Kramer et al. (1997)
MCF-7 cytosol	2',3,3',4',5-Pentachloro-4-biphenylol	192190-09-3	> 95	4.2				0.1	-1.000	Kramer et al. (1997)
GST-aERdef	2,3,3',4',5-Pentachloro-4-biphenylol	152969-11-4				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,3,3',4',5-Pentachloro-4-biphenylol	152969-11-4				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,3,3',4',5-Pentachloro-4-biphenylol	152969-11-4				10				Matthews and Zacharewski (2000)
GST-rtERdef	2',3,3',4',5-Pentachloro-4-biphenylol	192190-09-3				10				Matthews and Zacharewski (2000)
hERα	2',3,3',4',6'-Pentachloro-4-biphenylol	192190-10-6	> 98					0.13	-0.886	Kuiper et al. (1998) [method a]
hERβ	2',3,3',4',6'-Pentachloro-4-biphenylol	192190-10-6	> 98					0.12	-0.921	Kuiper et al. (1998) [method a]
MUC	2',3,3',4',6'-Pentachloro-4-biphenylol	192190-10-6	> 98			1000				Connor et al. (1997)
RUC	2',3,3',4',6'-Pentachloro-4-biphenylol	192190-10-6	> 98	35				0.041	-1.390	Connor et al. (1997)
hERα	2',3,3',5',6'-Pentachloro-4-biphenylol	189578-02-7	> 98					0.06	-1.222	Kuiper et al. (1998) [method a]
hERβ	2',3,3',5',6'-Pentachloro-4-biphenylol	189578-02-7	> 98					0.04	-1.398	Kuiper et al. (1998) [method a]
MUC	2',3',3',5',6'-Pentachloro-4-biphenylol	189578-02-7	> 98	6.7				0.031	-1.520	Connor et al. (1997)
RUC	2',3,3',5',6'-Pentachloro-4-biphenylol	189578-02-7	> 98	21				0.068	-1.170	Connor et al. (1997)
MCF-7 cytosol	2',3',4,4',5-Pentachloro-3-biphenylol	150975-81-8	> 95	3	1.2			2	0.301	Kramer et al. (1997)
MCF-7 cytosol	2,3',4,4',5-Pentachloro-3-biphenylol	170946-11-9	> 95	3.3	0.2			1	0.000	Kramer et al. (1997)
MCF-7 cytosol	2',3,4',5,5'-Pentachloro-4-biphenylol	149589-56-0						0.8	-0.969	Kramer et al. (1997)
MCF-7 cytosol	3,3',4',5,5'-Pentachloro-4-biphenylol	130689-92-8	> 95	5.1	1.8			0.02	-1.699	Kramer et al. (1997)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (µM)**	SD of IC_{50}**	HDT (µM)	Ki (µM)**	RBA***	log RBA***	Reference
RUC	Pentolame	150748-24-6		*20*				*0.04*	-1.398	Jaimez et al. (2000)
hERα-FP	Permethrin	52645-53-1	> 93			10				Saito et al. (2000)
MCF-7 cells	Phenanthrene	85-01-8				5				Arcaro et al. (1999)
RUC	4-Phenethylphenol	6335-83-7		44	6			0.002	-2.690	Blair et al. (2000)
MCF-7 cells	Phenol, 4,4'-[1,2-bis(methylene)-1,2-ethanediyl]bis-	107144-81-0						26	1.415	Stoessel and Leclerq (1986)
MCF-7 cytosol	Phenol, 4,4'-[1,2-bis(methylene)-1,2-ethanediyl]bis-	107144-81-0						20	1.301	Stoessel and Leclerq (1986)
MCF-7 cells	Phenol, 4-[7-(2 Dimethylamino)- ethoxy]-11-ethyldibenzo- [*b,f*]thiepin-10-yl]-	85850-74-4						0.3	-0.523	Stoessel and Leclerq (1986)
MCF-7 cytosol	Phenol, 4-[7-(2 Dimethylamino)- ethoxy]-11-ethyldibenzo- [*b,f*]thiepin-10-yl]-	85850-74-4						63	1.799	Stoessel and Leclerq (1986)
RUC	Phenol, 4-[7-(2 Dimethylamino)- ethoxy]-11-ethyldibenzo- [*b,f*]thiepin-10-yl]-	85850-74-4						63	1.799	Acton et al. (1983)
MCF-7 cells	Phenol, 4-[3-(2 dimethylamino)- ethoxy]-11-ethyldibenzo- [*b,f*]thioctin-12-yl)	85850-81-3						2.5	0.398	Stoessel and Leclerq (1986)
MCF-7 cytosol	Phenol, 4-[3-(2 dimethylamino)ethoxy]-11-ethyldibenzo[*b,f*]thioctin-12-yl)	85850-81-3						50	1.699	Stoessel and Leclerq (1986)
RUC	Phenol, 4-[3-(2 dimethylamino)- ethoxy]-11-ethyldibenzo- [*b,f*]thioctin-12-yl)	85850-81-3						52	1.716	Acton et al. (1983)
MCF-7 cells	Phenol, 4-[2-(2 dimethylamino)- ethoxy]- -6-ethyl-11,12-dihydro- dibenzo[*a,e*]-cycloocten-5-yl]-	85850-75-5						1.3	0.114	Stoessel and Leclerq (1986)
MCF-7 cytosol	Phenol, 4-[2-(2 dimethylamino)- ethoxy]- -6-ethyl-11,12-dihydro- dibenzo[*a,e*]-cycloocten-5-yl]-	85850-75-5						50	1.699	Stoessel and Leclerq (1986)
RUC	Phenol, 4-[2-(2 dimethylamino)- ethoxy]- -6-ethyl-11,12-dihydro- dibenzo[*a,e*]-cycloocten-5-yl]-	85850-75-5						50	1.699	Acton et al. (1983)
MCF-7 cells	Phenol, 3-[2- dimethylamino- ethoxy]- -10 ethyl- 4-hydroxy- phenyl dibenzo-[b,f]oxepin	85850-80-2						0.1	-1.000	Stoessel and Leclerq (1986)
MCF-7 cytosol	Phenol, 3-[2- dimethylamino- ethoxy]- -10 ethyl- 4-hydroxy- phenyl dibenzo-[b,f]oxepin	85850-80-2						6	0.778	Stoessel and Leclerq (1986)
RUC	Phenol, 3-[2- dimethylamino- ethoxy]- -10 ethyl- 4-hydroxy- phenyl dibenzo-[b,f]oxepin	85850-80-2						6.1	0.785	Acton et al. (1983)
MCF-7 cells	Phenol, 4-[1-[4-[2-(dimethyl- amino) ethoxy] phenyl]-2-phenyl-1-butenyl]-3-methyl-, (*E*)-	96474-35-0						0.4	-0.398	Stoessel and Leclerq (1986)
MCF-7 cytosol	Phenol, 4-[1-[4-[2-(dimethyl- amino) ethoxy] phenyl]-2-phenyl-1-butenyl]-3-methyl-, (*E*)-	96474-35-0						100	2.000	Stoessel and Leclerq (1986)
MCF-7 cells	Phenol, 4-(1, 2-diphenyl-1-butenyl)-	69967-79-9						2	0.301	Stoessel and Leclerq (1986)
MCF-7 cytosol	Phenol, 4-(1, 2-diphenyl-1-butenyl)-	69967-79-9						15	1.176	Stoessel and Leclerq (1986)
MCF-7 cells	Phenol, 4-(1*Z*)-1,2-diphenyl-1-butenyl)-	69967-80-2						0.4	-0.398	Stoessel and Leclerq (1986)
MCF-7 cytosol	Phenol, 4-(1*Z*)-1,2-diphenyl-1-butenyl)-	69967-80-2						1	0.000	Stoessel and Leclerq (1986)
MCF-7 cells	Phenol, 4-[2-Nitro-2-phenyl-1-[4-[2-(1-pyrrolidinyl)ethoxy]-phenyl]ethenyl]phenyl, (*E*)-	107144-84-3						2.1	0.322	Stoessel and Leclerq (1986)
MCF-7 cytosol	Phenol, 4-[2-Nitro-2-phenyl-1-[4-[2-(1-pyrrolidinyl)ethoxy]-phenyl]ethenyl]phenyl, (*E*)-	107144-84-3						100	2.000	Stoessel and Leclerq (1986)
MCF-7 cells	Phenol, 4,4'-(2-phenyl-1-butenylidene)bis-	91221-46-4						2	0.301	Stoessel and Leclerq (1986)
MCF-7 cytosol	Phenol, 4,4'-(2-phenyl-1-butenylidene)bis-	91221-46-4						100	2.000	Stoessel and Leclerq (1986)
RUC	Phenolphthalein	77-09-8		6.73	0.018			0.013	-1.870	Blair et al. (2000)
RUC	Phenolphthalin	81-90-3	99	425	75			0.0002	-3.670	Blair et al. (2000)
RUC	Phenol Red	143-74-8	95	160	60			0.001	-3.250	Blair et al. (2000)
hERα-FP	*d* -Phenothrin	26002-80-2	> 93			10				Saito et al. (2000)
RUC	2-Phenyl-3-(2-fluoro-4-hydroxyphenyl)-6-hydroxyindene							9.6	0.982	Anstead et al. (1990)
RUC	2-Phenyl-3-(2-fluorophenyl)-6-hydroxyindene							12	1.079	Anstead et al. (1990)
RUC	3-Phenyl-4'-hydroxy-2-phenylindene							0.017	-1.770	Anstead et al. (1989)
RUC	3-Phenyl-4'-hydroxy-2-phenylindene							0.36	-0.444	Anstead et al. (1989)
RUC	3-Phenyl-6-hydroxy-2-phenylindene							8.9	0.949	Anstead et al. (1989)
RUC	2-Phenyl-3-(2-methylphenyl)-6-hydroxyindene							11	1.041	Anstead et al. (1990)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (μM)**	SD of IC_{50}**	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
RUC	2-Phenyl-3-(4-methylphenyl)-6-hydroxyindene							1.7	0.230	Anstead et al. (1990)
RUC	2-Phenylphenol	90-43-7	99			100				Blair et al. (2000)
RUC	3-Phenylphenol	580-51-8	90	245	45			0.0004	-3.440	Blair et al. (2000)
MUC	4-Phenylphenol	92-69-3	> 98			5				Korach et al. (1988)
RUC	4-Phenylphenol	92-69-3	90	98	52			0.001	-3.040	Blair et al. (2000)
hERα	Phloretin	60-82-2						0.2	-0.699	Kuiper et al. (1998) [method a]
hERβ	Phloretin	60-82-2						0.7	-0.155	Kuiper et al. (1998) [method a]
RUC	Phloretin	60-82-2						0.069	-1.160	Fang et al. (2001)
hERα-FP	Prallethrin	23031-36-9	> 93			10				Saito et al. (2000)
hERα	Progesterone	57-83-0				100				Kuiper et al. (1997)
hERα	Progesterone	57-83-0				10				Kuiper et al. (1998) [method a]
hERβ	Progesterone	57-83-0				10				Kuiper et al. (1998) [method a]
MUC	Progesterone	57-83-0				10				Korach (1979)
rERβ	Progesterone	57-83-0				100				Kuiper et al. (1997)
RUC	Progesterone	57-83-0	99			1000				Blair et al. (2000)
RUC	Progesterone	57-83-0				2667				Laws et al. (2000)
RUC	Progesterone	57-83-0					1000	0.0003	-3.523	Waller et al. (1996)
RUC	Prolame	99876-41-2		*7*				*0.11*	-0.959	Jaimez et al. (2000)
RUC	Promegestone	34184-77-5	98			2667				Laws et al. (2000)
MUC	Promegestone	34184-77-5				10				Korach (1979)
RUC	Promegestone	34184-77-5					1.18	0.22	-0.658	Waller et al. (1996)
RUC	Prometon	1610-18-0				1000				Blair et al. (2000)
hERα-FP	Propazine	139-40-2	99.9			2000				Hanioka et al. (1999)
RUC	Propyl paraben	94-13-3	99	150	10			0.0006	-3.220	Blair et al. (2000)
hERα	Propylpyrazoletriol							49	1.690	Kraichely et al. (2000)
hERβ	Propylpyrazoletriol							0.12	-0.921	Kraichely et al. (2000)
RUC	Prunetin	552-59-0						0.0018	-2.740	Fang et al. (2001)
MUC	Pseudodiethylstilbestrol	39011-86-4		0.0011	0.0002			91	1.960	Korach et al. (1979)
MUC	Pseudodiethylstilbestrol	39011-86-4		1.1	0.2			145.5	2.160	Korach et al. (1985)
MCF-7 cells	Pyrene	129-00-0				5				Arcaro et al. (1999)
MCF-7 cells	Pyrrolidine, 1-[2-[4-[1-(4-methoxyphenyl)-2-nitro-2-phenylethenyl]phenoxy]ethyl]-, (*E*)	77413-87-7						0.07	-1.155	Stoessel and Leclerq (1986)
MCF-7 cytosol	Pyrrolidine, 1-[2-[4-[1-(4-methoxyphenyl)-2-nitro-2-phenylethenyl]phenoxy]ethyl]-, (*E*)	77413-87-7						11	1.041	Stoessel and Leclerq (1986)
hERα	Quercetin	117-39-5						0.01	-2.000	Kuiper et al. (1998) [method a]
hERβ	Quercetin	117-39-5						0.04	-1.398	Kuiper et al. (1998) [method a]
MCF-7 cytosol	Quercetin	117-39-5				25				Miodini et al. (1999)
RUC	Quercetin	117-39-5				100				Fang et al. (2001)
GST-aERdef	Quercetin	117-39-5		19	2			0.016	-1.796	Matthews et al. (2000)
GST-cERdef	Quercetin	117-39-5		82	22			0.0039	-2.409	Matthews et al. (2000)
GST-hERαdef	Quercetin	117-39-5				*100*			n.a	Matthews et al. (2000)
GST-mERαdef	Quercetin	117-39-5				*100*				Matthews et al. (2000)
GST-rtERdef	Quercetin	117-39-5		8	2			0.042	-1.377	Matthews et al. (2000)
MCF-7 cells	6-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-	107144-82-1						22	1.342	Stoessel and Leclerq (1986)
MCF-7 cytosol	6-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-	107144-82-1						33	1.519	Stoessel and Leclerq (1986)
MCF-7 cells	7-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-	107144-83-2						3	0.477	Stoessel and Leclerq (1986)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (µM)**	SD of IC_{50}**	HDT (µM)	Ki (µM)**	RBA***	log RBA***	Reference
MCF-7 cytosol	7-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-	107144-83-2						9	0.954	Stoessel and Leclerq (1986)
hERα	Raloxifene	84449-90-1						69	1.839	Kuiper et al. (1998) [method a]
hERα	Raloxifene	84449-90-1		0.0018				51.7	1.700	Kuiper et al. (1998) [method b]
hERβ	Raloxifene	84449-90-1						16	1.204	Kuiper et al. (1998) [method a]
hERβ	Raloxifene	84449-90-1		0.0041				25.9	1.410	Kuiper et al. (1998) [method b]
hERα	Raloxifene hydrochloride	82640-04-8		0.0008				62.5	1.796	Arcaro et al. (2000)
RUC	Resveratrol	501-36-0		*0.001*				*300*	2.477	Ashby et al. (1999)
RUC	Rutin	153-18-4						0.000082	-4.090	Fang et al. (2001)
GST-aERdef	Simazine	122-34-9				100				Matthews et al. (2000)
GST-cERdef	Simazine	122-34-9				100				Matthews et al. (2000)
GST-hERαdef	Simazine	122-34-9				100				Matthews et al. (2000)
GST-mERαdef	Simazine	122-34-9				100				Matthews et al. (2000)
GST-rtERdef	Simazine	122-34-9				100				Matthews et al. (2000)
hERα-FP	Simazine	122-34-9	99.4			2000				Hanioka et al. (1999)
RUC	Simazine	122-34-9	99			33.3				Blair et al. (2000)
GST-aERdef	β-Sitosterol	83-46-5				100				Matthews et al. (2000)
GST-cERdef	β-Sitosterol	83-46-5				100				Matthews et al. (2000)
GST-hERαdef	β-Sitosterol	83-46-5				100			n.a	Matthews et al. (2000)
GST-mERαdef	β-Sitosterol	83-46-5				100				Matthews et al. (2000)
GST-rtERdef	β-Sitosterol	83-46-5				100				Matthews et al. (2000)
hERα	β-Sitosterol	83-46-5				100				Kuiper et al. (1997)
rERβ	β-Sitosterol	83-46-5				100				Kuiper et al. (1997)
RUC	β-Sitosterol	83-46-5				*1000*				Fang et al. (2001)
RUC	4,4'-Stilbenediol	659-22-3		0.32	0.09			0.281	-0.550	Blair et al. (2000)
RUC	4-Stilbenol	3839-46-1				100				Blair et al. (2000)
RUC	Suberic acid	505-48-6	99			100				Blair et al. (2000)
GST-aERdef	Tamoxifen	10540-29-1		0.03	0.003			10	1.000	Matthews et al. (2000)
GST-cERdef	Tamoxifen	10540-29-1		0.021	0.001			16	1.204	Matthews et al. (2000)
GST-hERαdef	Tamoxifen	10540-29-1		0.028	0.004			11	1.041	Matthews et al. (2000)
GST-mERαdef	Tamoxifen	10540-29-1		0.026	0.001			10	1.000	Matthews et al. (2000)
GST-rtERdef	Tamoxifen	10540-29-1		0.013	0.001			25	1.398	Matthews et al. (2000)
hERα	Tamoxifen	10540-29-1					0.0034	7	0.845	Kuiper et al. (1997)
hERα	Tamoxifen	10540-29-1						4	0.602	Kuiper et al. (1998) [method a]
hERα	Tamoxifen	10540-29-1		0.17				2.94	0.47	Morito et al. (2001)
hERα-FP	Tamoxifen	10540-29-1		0.423				3.1	0.491	Bolger et al. (1998)
hERα-FP	Tamoxifen	10540-29-1		0.189				2.96	0.471	Parker et al. (2000)
hERβ	Tamoxifen	10540-29-1						3	0.477	Kuiper et al. (1998) [method a]
hERβ	Tamoxifen	10540-29-1		0.3				1.67	0.22	Morito et al. (2001)
MUC	Tamoxifen	10540-29-1	99	*0.0275*				*6.55*	*0.816*	Shelby et al. (1996)
RBC	Tamoxifen	10540-29-1	96	0.12				0.017	-1.770	Andersen et al. (1999)
rERβ	Tamoxifen	10540-29-1					0.0025	6	0.778	Kuiper et al. (1997)
RUC	Tamoxifen	10540-29-1						5.9	0.771	Acton et al. (1983)
RUC	Tamoxifen	10540-29-1		6				0.13	-0.523	Allen et al. (1980)
RUC	Tamoxifen	10540-29-1		0.063				0.3	-0.523	Liu et al. (1994)
RUC	Tamoxifen	10540-29-1						6	0.778	Qian and Abul-Hajj (1990)
MCF-7 cells	Tamoxifen	10540-29-1						0.06	-1.222	Stoessel and Leclerq (1986)
MCF-7 cytosol	Tamoxifen	10540-29-1						1	0.000	Stoessel and Leclerq (1986)
RUC	Tamoxifen citrate	54965-24-1		0.0555	0.0005			1.62	0.210	Blair et al. (2000)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (μM)**	SD of IC_{50}**	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
RUC	Taxifolin	480-18-2				100				Fang et al. (2001)
hERα	Testosterone	58-22-0				100				Kuiper et al. (1997)
hERα	Testosterone	58-22-0				10				Kuiper et al. (1998) [method a]
hERα-FP	Testosterone	58-22-0		35	0.5			0.04	-1.398	Nikov et al. (2000)
hERβ	Testosterone	58-22-0				10				Kuiper et al. (1998) [method a]
MUC	Testosterone	58-22-0				10				Korach (1979)
RBC	Testosterone	58-22-0	100			100				Andersen et al. (1999)
rERβ	Testosterone	58-22-0				100				Kuiper et al. (1997)
RUC	Testosterone	58-22-0	98			1000				Blair et al. (2000)
RUC	Testosterone	58-22-0					28.97	0.01	-2.000	Waller et al. (1996)
GST-aERdef	2,2',3,3'-Tetrachlorobiphenyl	3844-93-8				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3,3'-Tetrachlorobiphenyl	3844-93-8				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,3'-Tetrachlorobiphenyl	3844-93-8				10				Matthews and Zacharewski (2000)
GST-aERdef	2,2',3,4,-Tetrachlorobiphenyl	52663-59-9				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3,4,-Tetrachlorobiphenyl	52663-59-9				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,4,-Tetrachlorobiphenyl	52663-59-9		10				0.024	-1.620	Matthews and Zacharewski (2000)
GST-aERdef	2,2',3,6-Tetrachlorobiphenyl	41464-47-5				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',3,6-Tetrachlorobiphenyl	41464-47-5				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',3,6-Tetrachlorobiphenyl	41464-47-5		10				0.031	-1.509	Matthews and Zacharewski (2000)
RUC	2,2',4,4'-Tetrachlorobiphenyl	2437-79-8	98.4			100				Blair et al. (2000)
GST-aERdef	2,2',4,4'-Tetrachlorobiphenyl	2437-79-8				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',4,4'-Tetrachlorobiphenyl	2437-79-8				10			n.a	Matthews and Zacharewski (2000)
GST-rtERdef	2,2',4,4'-Tetrachlorobiphenyl	2437-79-8		10				0.031	-1.509	Matthews and Zacharewski (2000)
GST-aERdef	2,2',4,5'-Tetrachlorobiphenyl	41464-40-8				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',4,5'-Tetrachlorobiphenyl	41464-40-8				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',4,5'-Tetrachlorobiphenyl	41464-40-8				10				Matthews and Zacharewski (2000)
GST-aERdef	2,2',4,6'-Tetrachlorobiphenyl	68194-04-7				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',4,6'-Tetrachlorobiphenyl	68194-04-7				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',4,6'-Tetrachlorobiphenyl	68194-04-7		10				0.031	-1.509	Matthews and Zacharewski (2000)
hERα	2,2',5,5'-Tetrachlorobiphenyl	35693-99-3	≥ 99%			50				Vakharia and Gierthy (2000)
GST-aERdef	2,2',6,6'-Tetrachlorobiphenyl	15968-05-5				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',6,6'-Tetrachlorobiphenyl	15968-05-5				10			n.a	Matthews and Zacharewski (2000)
GST-rtERdef	2,2',6,6'-Tetrachlorobiphenyl	15968-05-5		10				0.031	-1.509	Matthews and Zacharewski (2000)
hERα	2,2',6,6'-Tetrachlorobiphenyl	15968-05-5	≥ 99			100				Arcaro et al. (1999)
hERα	2,2',6,6'-Tetrachlorobiphenyl	15968-05-5	≥ 99%			50				Vakharia and Gierthy (2000)
hERβ	2,2',6,6'-Tetrachlorobiphenyl	15968-05-5	≥ 99			100				Arcaro et al. (1999)
GST-aERdef	2,3,3',5'-Tetrachlorobiphenyl	41464-49-7				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,3,3',5'-Tetrachlorobiphenyl	41464-49-7				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,3,3',5'-Tetrachlorobiphenyl	41464-49-7				10				Matthews and Zacharewski (2000)
GST-aERdef	2,3,4,4'-Tetrachlorobiphenyl	33025-41-1				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,3,4,4'-Tetrachlorobiphenyl	33025-41-1				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,3,4,4'-Tetrachlorobiphenyl	33025-41-1				10				Matthews and Zacharewski (2000)
GST-aERdef	2,3',4',5-Tetrachlorobiphenyl	32598-11-1				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,3',4',5-Tetrachlorobiphenyl	32598-11-1				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,3',4',5-Tetrachlorobiphenyl	32598-11-1				10				Matthews and Zacharewski (2000)
GST-aERdef	2,3',4,5'-Tetrachlorobiphenyl	73575-52-7				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,3',4,5'-Tetrachlorobiphenyl	73575-52-7				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,3',4,5'-Tetrachlorobiphenyl	73575-52-7				10				Matthews and Zacharewski (2000)
GST-aERdef	2,4,4',5-Tetrachlorobiphenyl	32690-93-0				10				Matthews and Zacharewski (2000)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (µM)**	SD of IC_{50}**	HDT (µM)	Ki (µM)**	RBA***	log RBA***	Reference
GST-hERαdef	2,4,4',5-Tetrachlorobiphenyl	32690-93-0				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,4,4',5-Tetrachlorobiphenyl	32690-93-0				10				Matthews and Zacharewski (2000)
hERα	3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	≥ 99%			50				Vakharia and Gierthy (2000)
GST-aERdef	3,3',4,4'-Tetrachlorobiphenyl	32598-13-3				10				Matthews and Zacharewski (2000)
GST-hERαdef	3,3',4,4'-Tetrachlorobiphenyl	32598-13-3				10				Matthews and Zacharewski (2000)
GST-rtERdef	3,3',4,4'-Tetrachlorobiphenyl	32598-13-3				10				Matthews and Zacharewski (2000)
RUC	3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	99			300				Blair et al. (2000)
GST-aERdef	3,3',4,5-Tetrachlorobiphenyl	70362-49-1				10				Matthews and Zacharewski (2000)
GST-hERαdef	3,3',4,5-Tetrachlorobiphenyl	70362-49-1				10				Matthews and Zacharewski (2000)
GST-rtERdef	3,3',4,5-Tetrachlorobiphenyl	70362-49-1				10				Matthews and Zacharewski (2000)
MUC	2',3',5',6'-Tetrachloro-4,4'-biphenyldiol	100702-98-5	> 98	5.0				0.020	-1.699	Korach et al. (1988)
MUC	3,3',5,5'-Tetrachloro-4,4'-biphenyldiol	13049-13-3	> 98	1.354				0.074	-1.130	Korach et al. (1988)
MUC	3,3',5,5'-Tetrachloro-4,4'-biphenyldiol	13049-13-3					1.95	0.13	-0.880	Waller et al. (1996)
MCF-7 cytosol	3,3',5,5'-Tetrachloro-4,4'-biphenyldiol	13049-13-3	> 95	3.7	0.3			0.4	-0.398	Kramer et al. (1997)
RUC	3,3',5,5'-Tetrachloro-4,4'-biphenyldiol	13049-13-3	95	160	10			0.001	-3.250	Blair et al. (2000)
hERα	2,2',4',6'-Tetrachloro-4-biphenylol	150304-08-8	> 98					0.3	-0.523	Kuiper et al. (1998) [method a]
hERβ	2,2',4',6'-Tetrachloro-4-biphenylol	150304-08-8	> 98					0.5	-0.301	Kuiper et al. (1998) [method a]
MUC	2,2',4',6'-Tetrachloro-4-biphenylol	150304-08-8	> 98	12				0.018	-1.740	Connor et al. (1997)
RUC	2,2',4',6'-Tetrachloro-4-biphenylol	150304-08-8	> 98	2700				0.00053	-3.275	Connor et al. (1997)
hERα	2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8		*0.2*				*0.3*	*-0.520*	Arcaro et al. (1999)
hERα	2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8		*0.2*				*0.3*	*-0.520*	Arcaro et al. (1999)
hERβ	2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8		*1.995*				*0.25*	*-0.600*	Arcaro et al. (1999)
hERβ	2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8		*1.995*				*0.25*	*-0.600*	Arcaro et al. (1999)
GST-aERdef	2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8		0.5	0.2			0.50	-0.301	Matthews and Zacharewski (2000)
GST-hERαdef	2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8		0.5	0.02			0.48	-0.319	Matthews and Zacharewski (2000)
GST-rtERdef	2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8		0.3	0.1			1.03	0.013	Matthews and Zacharewski (2000)
hERα	2,2',6,6'-tetrachloro-4-biphenylol	219952-18-8	95 - 99	0.5				0.20	-0.700	Vakharia and Gierthy (2000)
MCF-7 cytosol	2',3',4',5'-Tetrachloro-3-biphenylol	67651-37-0	> 95	4.3	0.7			0.1	-1.000	Kramer et al. (1997)
GST-aERdef	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7		0.5	0.2			0.50	-0.301	Matthews and Zacharewski (2000)
hERα	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	> 98					3.4	0.531	Kuiper et al. (1998) [method a]
hERβ	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	> 98					7.2	0.857	Kuiper et al. (1998) [method a]
MUC	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	> 98	0.0950				1.05	0.021	Korach et al. (1988)
MUC	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	> 98	0.990				1.11	0.046	Ramamoorthy et al. (1997b)
MUC	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7					0.0452	5.75	0.760	Waller et al. (1996)
MCF-7 cytosol	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	> 95	2.8	0.6			3.2	0.505	Kramer et al. (1997)
GST-hERαdef	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7		0.1	0.02			2.4	0.380	Matthews and Zacharewski (2000)
GST-rtERdef	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7		0.27	0.02			1.15	0.061	Matthews and Zacharewski (2000)
RUC	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	95	0.395	0.015			0.23	-0.640	Blair et al. (2000)
hERα	2',3,4',6'-Tetrachloro-4-biphenylol	189578-00-5	> 98					0.18	-0.745	Kuiper et al. (1998) [method a]
hERβ	2',3,4',6'-Tetrachloro-4-biphenylol	189578-00-5	> 98					0.23	-0.638	Kuiper et al. (1998) [method a]
MUC	2',3,4',6'-Tetrachloro-4-biphenylol	189578-00-5	> 98			*1000*				Connor et al. (1997)
RUC	2',3,4',6'-Tetrachloro-4-biphenylol	189578-00-5	> 98			*1000*				Connor et al. (1997)
MCF-7 cells	2,3,7,8,-Tetrachlorodibenzo-*p* -dioxin	1746-01-6				5				Arcaro et al. (1999)
hERα	Tetrahydrochrysene	104460-72-2						3	0.477	Meyers et al. (1999)
hERβ	Tetrahydrochrysene	104460-72-2						6.5	0.813	Meyers et al. (1999)
hERβ	(*rac*)-Tetrahydrochrysene						0.0036	25	1.398	Sun et al. (1999)
hERα	(*rac*)-Tetrahydrochrysene						0.013	2.5	0.398	Sun et al. (1999)
hERα	(*R* ,*R*)-Tetrahydrochrysene						0.009	3.6	0.556	Sun et al. (1999)
hERβ	(*R* ,*R*)-Tetrahydrochrysene						0.0036	25	1.398	Sun et al. (1999)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (μM)**	SD of IC_{50}**	HDT (μM)	Ki (μM)**	RBA***	log RBA***	Reference
hERα	(*S* ,*S*)-Tetrahydrochrysene						0.039	0.83	-0.081	Sun et al. (1999)
hERβ	(*S* ,*S*)-Tetrahydrochrysene						0.07	1.3	0.114	Sun et al. (1999)
RUC	2,2',4,4'-Tetrahydroxybenzil	5394-98-9		0.43	0			0.209	-0.680	Blair et al. (2000)
MCF-7 cells	Tetramethylhexestrol	74385-27-6						2	0.301	Stoessel and Leclerq (1986)
MCF-7 cytosol	Tetramethylhexestrol	74385-27-6						1.5	0.176	Stoessel and Leclerq (1986)
RUC	Thalidomide	50-35-1	99			1000				Blair et al. (2000)
RUC	Toremifene citrate	89778-27-8		0.065	0.005			1.38	0.140	Blair et al. (2000)
RUC	Tosyl Nonylphenol (mixed branched isomers)			*80*				*0.006*	-2.204	Elsby et al. (2000)
hERα	Toxaphene	8001-35-2	95 - 99			10				Arcaro et al. (2000)
MCF-7 cytosol	Toxaphene	8001-35-2	Technical grade	470	38			0.00032	-3.495	Soto et al. (1995)
MUC	Toxaphene	8001-35-2	Technical grade			10				Ramamoorthy et al. (1997a)
hERα	Triaryl-pyrazole						0.00054	60	1.778	Sun et al. (1999)
hERβ	Triaryl-pyrazole						0.0051	18	1.255	Sun et al. (1999)
GST-aERdef	2,2',5-Trichlorobiphenyl	37680-65-2				10				Matthews and Zacharewski (2000)
GST-hERαdef	2,2',5-Trichlorobiphenyl	37680-65-2				10				Matthews and Zacharewski (2000)
GST-rtERdef	2,2',5-Trichlorobiphenyl	37680-65-2				10				Matthews and Zacharewski (2000)
hERα	2,4,6-Trichlorobiphenyl	35693-92-6	≥ 99%			100				Vakharia and Gierthy (1999)
hERα	2',4',6'-Trichloro-4-biphenylol	14962-28-8	> 98					2.4	0.380	Kuiper et al. (1998) [method a]
hERβ	2',4',6'-Trichloro-4-biphenylol	14962-28-8	> 98					4.7	0.672	Kuiper et al. (1998) [method a]
MUC	2',4',6'-Trichloro-4-biphenylol	14962-28-8					0.048	5.37	0.730	Waller et al. (1996)
hERα	2',4',6'-Trichloro-4-biphenylol	14962-28-8	≥ 99%	0.079				0.75	-0.127	Vakharia and Gierthy (1999)
MCF-7 cytosol	2',4',6'-Trichloro-4-biphenylol	14962-28-8	> 95	2.5	1.2			6.3	0.799	Kramer et al. (1997)
MUC	2',4',6'-Trichloro-4-biphenylol	14962-28-8	> 98	0.0420				2.38	0.377	Korach et al. (1988)
MUC	2',4',6'-Trichloro-4-biphenylol	14962-28-8	> 98	3.4				0.32	-0.490	Ramamoorthy et al. (1997b)
MCF-7 cytosol	3,3',4-Trichloro-4-biphenylol	124882-64-0	> 95	3.8	0.1			0.3	-0.523	Kramer et al. (1997)
MUC	3,4',5-Trichloro-4-biphenylol	4400-06-0	> 98	1.0000				0.10	-1.000	Korach et al. (1988)
RUC	2,4,5-Trichlorophenoxyacetic acid	93-76-5	98			1000				Blair et al. (2000)
RBC	Triethylamine, 2-[*p* -[6-methoxy-2-phenyl-3-inden-3-yl)phenoxy] hydrochloride	64-96-0						0.00059	-3.229	Korenman (1970)
hERα-FP	Triethylene glycol dimethacrylate	109-16-0	95			5000				Hashimoto et al. (2000)
RUC	4,2',4'-Trihydroxychalcone	961-29-5						0.054	-1.270	Fang et al. (2001)
RUC	3,6,4'-Trihydroxyflavone	253195-19-6						0.45	-0.350	Fang et al. (2001)
RUC	6,7,4'-Trihydroxyisoflavone	17817-31-1				100				Fang et al. (2001)
RUC	7,3',4'-Trihydroxyisoflavone	485-63-2						0.0045	-2.350	Fang et al. (2001)
RUC	1,1,2-Triphenylbut-1-ene	63019-13-6						0.01	-2.000	Jordan et al. (1986)
RUC	Triphenylethylene	58-72-0	99	54.5	5.5			0.002	-2.780	Blair et al. (2000)
RUC	Triphenyl phosphate	115-86-6	99			100				Blair et al. (2000)
MCF-7 cells	Tris-4-(chlorophenyl)methane	27575-78-6	94	*0.4*				*0.1*	-1.00	Lascombe et al. (2000)
MCF-7 cells	Tris-4-(chlorophenyl)methanol	30100-80-8	94	*0.4*				*0.1*	-1.00	Lascombe et al. (2000)
RUC	Vanillin	121-33-5	99			100				Blair et al. (2000)
RUC	Vinclozolin	50471-44-8	98.2			100				Blair et al. (2000)
RUC	Vinclozolin	50471-44-8					1000	0.0003	-3.523	Waller et al. (1996)
RUC	α-Zearalanol	26538-44-3						30	1.480	Fang et al. (2001)
hERα	β-Zearalanol	42422-68-4					0.0008	16	1.200	Kuiper et al. (1997)
rERβ	β-Zearalanol	42422-68-4					0.0009	14	1.146	Kuiper et al. (1997)
RUC	β-Zearalanol	42422-68-4						0.64	-0.190	Fang et al. (2001)
RUC	Zearalanone	5975-78-0						2.1	0.320	Fang et al. (2001)
RUC	α-Zearalenol	36455-72-8						43	1.630	Fang et al. (2001)
GST-aERdef	α-Zearalenol	36455-72-8		0.0086	0.0021			36	1.556	Matthews et al. (2000)

Data Sorted by Substance and Assay

Assay Type*	Substance	CASRN†	Purity (%)††	IC_{50} (µM)**	SD of IC_{50}**	HDT (µM)	Ki (µM)**	RBA***	log RBA***	Reference
GST-cERdef	α-Zearalenol	36455-72-8		0.0046	0.0009			70	1.845	Matthews et al. (2000)
GST-hERαdef	α-Zearalenol	36455-72-8		0.0061	0.0002			48	1.681	Matthews et al. (2000)
GST-mERαdef	α-Zearalenol	36455-72-8		0.0051	0.0005			53	1.724	Matthews et al. (2000)
GST-rtERdef	α-Zearalenol	36455-72-8		0.0013	0.0001			267	2.427	Matthews et al. (2000)
RUC	β-Zearalenol	71030-11-0						0.2	-0.700	Fang et al. (2001)
GST-aERdef	β-Zearalenol	71030-11-0		0.073	0.018			4.2	0.623	Matthews et al. (2000)
GST-cERdef	β-Zearalenol	71030-11-0		0.014	0.001			23	1.362	Matthews et al. (2000)
GST-hERαdef	β-Zearalenol	71030-11-0		0.023	0.003			13	1.114	Matthews et al. (2000)
GST-mERαdef	β-Zearalenol	71030-11-0		0.024	0.016			11	1.041	Matthews et al. (2000)
GST-rtERdef	β-Zearalenol	71030-11-0		0.0037	0.0003			91	1.959	Matthews et al. (2000)
GST-aERdef	Zearalenone	17924-92-4		0.027	0.003			12	1.079	Matthews et al. (2000)
GST-cERdef	Zearalenone	17924-92-4		0.0099	0.0011			33	1.519	Matthews et al. (2000)
GST-hERαdef	Zearalenone	17924-92-4		0.031	0.003			9.3	0.968	Matthews et al. (2000)
GST-mERαdef	Zearalenone	17924-92-4		0.023	0.005			12	1.079	Matthews et al. (2000)
GST-rtERdef	Zearalenone	17924-92-4		0.0041	0.0008			82	1.914	Matthews et al. (2000)
hERα	Zearalenone	17924-92-4						7	0.845	Kuiper et al. (1998) [method a]
hERα	Zearalenone	17924-92-4		0.009				10	1.000	Kuiper et al. (1998) [method b]
hERα-FP	Zearalenone	17924-92-4		0.059	0.0008			22	1.342	Nikov et al. (2000)
hERβ	Zearalenone	17924-92-4						5	0.699	Kuiper et al. (1998) [method a]
hERβ	Zearalenone	17924-92-4		0.0058				18	1.255	Kuiper et al. (1998) [method b]
MCF-7 cytosol	Zearalenone	17924-92-4		*0.007*				18	1.255	Dodge et al. (1996)
RUC	Zearalenone	17924-92-4					0.0059	44.07	1.644	Waller et al. (1996)

[a]Numbers in italics were estimated from a graphical representation of the data.

*GST-aERdef = glutathione-*S* -transferase fusion proteins consisting of the "def" domain of the lizard (anole); GST-cERdef = glutathione-*S* -transferase fusion proteins consisting of the "def" domain of the chicken; GST-hERαdef = glutathione-*S* -transferase fusion proteins consisting of the "def" domain of the human ERα receptor; GST-mERαdef = glutathione-*S* -transferase fusion proteins consisting of the "def" domain of the mouse ERα receptor; GST-rtERdef = glutathione-*S* -transferase fusion proteins consisting of the "def" domain of the rainbow trout; hERα = semi-purified human ERα protein; hERα-FP = semi-purified human ERα measured using fluorescence polarization; hERβ = semi-purified human ERβ protein; MCF-7 cells = intact MCF-7 cells; MCF-7 cytosol = cytosol from human adenocarcinoma cells; MUC = mouse uterine cytosol; RBC = rabbit uterine cytosol; rERβ = semi-purified rat ERβ protein; RUC = rat uterine cytosol. HDT = highest dose tested; IC_{50} = concentration of the test substance that induces a 50% decrease in binding by the reference estrogen; Ki = dissociation constant of a receptor-ligand complex; RBA = relative binding affinity = (IC50 test substance/IC50 reference estrogen) x100; SD = standard deviation.

**Empty cells indicate that an IC_{50} or K_i could not be obtained, in which case the HDT was reported, or that the IC_{50} or K_i was not reported but instead the RBA was reported.

*** Empty cells indicate that an RBA was not provided or could not be calculated because an IC_{50} or K_i could not be obtained. Thus no log RBA could be determined.

† Empty cells indicate that no CASRN could be found.

†† Empty cells indicate that no information was provided in the publication.

Appendix D2

Substances Tested in the *In Vitro* ER Binding Assays

References

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References

Acton, D., Hill, G., Tait, B.S. (1983) Tricyclic triarylethylene antiestrogens: dibenz[*b,f*]oxepins, dibenzo[*b,f*]thiepins, dibenzo[*a,e*]cyclooctenes, and dibenzo[*b,f*]thiocins. J Med Chem 26:1131-1137.

Allen, K.E., Clark, E.R., Jordan, V.C. (1980) Evidence for the metabolic activation of non-steroidal antioestrogens: a study of structure-activity relationships. Br J Pharmacol 71:83-91.

Andersen, H.R., Anderson, A.-M., Arnold, S.F., Autrup, H., Barfoed, M., Beresford, N.A., Bjerregaard, P., Christiansen, L.B., Gissel, B., Hummel, R., Jorgensen, E.B., Korsgaard, B., Guevel, R.L., Leffers, H., McLachlan, J., Moller, A., Nielsen, J.B., Olea, N., Oles-Karasko, A., Pakdel, F., Pedersen, K.L., Perez, P., Skakkeboek, N.E., Sonnenschein, C., Soto, A.M., Sumpter, J.P., Thorpe, S.M., Grandjean, P. (1999) Comparison of short-term estrogenicity tests for identification of hormone-disrupting chemicals. Environ Health Perspect 107 (Suppl. 1):89-108.

Anstead, G.M., Peterson, C.S., Pinney, K.G., Wilson, S.R., Katzenellenbogen, J.A. (1990) Torsionally and hydrophobically modified 2,3-diarylindenes as estrogen receptor ligands. J Med Chem 33:2726-2734.

Anstead, G.M., Wilson, S.R., Katzenellenbogen, J.A. (1989) 2-Arylindenes and 2-arylindenones: Molecular structures and considerations in the binding orientation of unsymmetrical nonsteroidal ligands to the estrogen receptor. J Med Chem 32:2163-2171.

Arcaro, K.F., Yang, Y., Vakharia, D.D., Gierthy, J.F. (2000) Toxaphene is antiestrogenic in a human breast-cancer cell assay. J Toxicol Environ Health 59:197-210.

Arcaro, K.F., Yi, L., Seegal, R.F., Vakharia, D.D., Yang, Y., Spink, D.C., Brosch, K., Gierthy, J.F. (1999) 2,2',6,6'-Tetrachlorobiphenyl is estrogenic *in vitro* and *in vivo*. J Cell Biochem 72:94-102.

Ashby, J., Tinwell, H.W., Pennie, W., Brooks, A.N., Lefevre, P.A., Beresford, N., Sumpter, J.P. (1999) Partial and weak oestrogenicity of the red wine constituent resveratrol: Consideration of its superagonist activity in MCF-7 cells and its suggested cardiovascular protective effects. J Appl Toxicol 19:39-45.

Blair, R.M., Fang, H., Branham, W.S., Hass, B.S., Dial, S.L., Moland, C.L., Tong, W., Shi, L., Perkins, R., Sheehan, D.M. (2000) The estrogen receptor relative binding affinities of 188 natural and xenochemicals: Structural diversity of ligands. Toxicol Sci 54:138-153.

Bolger, R., Wiese, T.E., Ervin, K., Nestich, S., Checovich, W. (1998) Rapid screening of environmental chemicals for estrogen receptor binding capacity. Environ Health Perspect 106:551-557.

Brooks, S.C., Wappler, N.L., Corombos, J.D., Doherty, L.M. (1987) Estrogen structure-receptor function relationships. In: Recent Advances in Steroid Hormone Action (Moudgil, V.K., ed). Berlin and New York:De Gruyter, 443-466.

Chae, K., Gibson, M.K., Korach, K.S. (1991) Estrogen receptor stereochemistry: Ligand binding orientation and influence on biological activity. Mol Pharmacol 40:806-811.

Connor, K., Ramamoorthy, K., Moore, M., Mustain, M., Chen, I., Safe, S., Zacharewski, T., Gillesby, B., Joyeux, A., Balaguer, P. (1997) Hydroxylated polychlorinated biphenyls (PCBs) as estrogens and antiestrogens: Structure-activity relationships. Toxicol Appl Pharmacol 145:111-123.

Dodge, J.A., Glasebrook, A.L., Magee, D.E., Phillips, D.L., Sato, M., Short, L.L., Bryant, H.U. (1996) Environmental estrogens: Effects on cholesterol lowering and bone in the ovariectomized rat. J Steroid Biochem Mol Biol 59:155-161.

Elsby, R., Ashby, J., Sumpter, J.P., Brooks, A.N., Pennie, W.D., Maggs, J.L., Lefevre, P.A., Odum, J., Beresford, N., Paton, D., Park, B.K. (2000) Obstacles to the prediction of estrogenicity from chemical structure: Assay-mediated metabolic transformation and the apparent promiscuous nature of the estrogen receptor. Biochem Pharmacol 60:1519-1530.

Fang, H., Tong, W., Shi, L.M., Blair, R., Perkins, R., Branham, W., Hass, B.S., Xie, Q., Dial, S.L., Moland, C.L., Sheehan, D.M. (2001) Structure-activity relationships for a large diverse set of natural, synthetic, and environmental estrogens. Chem Res Toxicol 14:280-294.

Fertuck, K.C., Matthews, J.B., Zacharewski, T.R. (2001) Hydroxylated benzo[*a*]pyrene metabolites are responsible for *in vitro* estrogen receptor-mediated gene expression induced by benzo[*a*]pyrene, but do not elicit uterotrophic effects *in vivo*. Toxicol Sci 59:231-240.

Fielden, M.R., Chen, I., Chittim, B., Safe, S.H., Zacharewski, T.R. (1997) Examination of the estrogenicity of 2,4,6,2',6'-pentachlorobiphenyl (PCB 104), its hydroxylated metabolite 2,4,6,2'6'-pentachloro-4-biphenylol (HO-PCB 104), and a further chlorinated derivative, 2,4,6,2',4',6'-hexachlorobiphenyl (PCB 155). Environ Health Perspect 105:1238-1248.

Gabbard, R.B., Segaloff, A. (1983) Structure-activity relationships of estrogens. Effects of 14-dehydrogenaton and axial methyl groups at C-7, C-9 and C-11. Steroids 41:791-805.

Gaido, K.W., Leonard, L.S., Maness, S.C., Hall, J.M., McDonnell, D.P., Saville, B., Safe, S.H. (1999) Differential interaction of the methoxychlor metabolite 2,2-bis-(*p*-hydroxyphenyl)-1,1,-trichloroethane with estrogen receptors α and β. Endocrinology 140:5746-5753

Hanioka, N., Jinno, H., Tanaka-Kagawa, T., Nishimura, T., Ando, M. (1999) *In vitro* metabolism of simazine, atrazine and propazine by hepatic cytochrome P450 enzymes of rat, mouse and guinea pig, and oestrogenic activity of chlorotriazines and their main metabolites. Xenobiotica 29:1213-1226.

Hashimoto, Y., Moriguchi, Y., Oshima, H., Nishikawa, J., Nishihara, T., Nakamura, M. (2000) Estrogenic activity of chemicals for dental and similar use *in vitro*. J Mater Sci-Mater Med 11:465-468.

Jaimez, R., Cooney, A., Jackson, K., Lemus, A.E., Limini, C., Cardenas, M., Garcia, R., Silva, G., Larrea, F. (2000) *In vivo* estrogen bioactivities and *in vitro* estrogen receptor binding and transcriptional activities of anticoagulant synthetic 17ß-aminoestrogens. J Biochem Mol Biol 73:59-66.

Jordan, V.C., Koch, R., Mittal, S., Schneider, M.R. (1986) Oestrogenic and antioestrogenic actions in a series of triphenylbut-1-enes: Modulation of prolactin synthesis in vitro. Br J Pharmacol 87:217-223.

Klotz, D.M., Beckman, B.S., Hill, S.M., McLachlan, J.A., Walters, M.R., Arnold, S.F. (1996) Identification of environmental chemicals with estrogenic activity using a combination of *in vitro* assays. Environ Health Perspect 104:1084-1089.

Korach, K.S. (1979) Estrogen action in the mouse uterus: Characterization of the cytosol and nuclear receptor systems. Endocrinology 104:1324-1332.

Korach, K.S., Chae, K., Levy, L.A., Duax, W.L., Sarver, P.J. (1989) Diethylstilbestrol metabolites and analogs -- Stereochemical probes for the estrogen receptor binding site. J Biol Chem 264:5642-5647.

Korach, K.S., Metzler, M., McLachlan, J.A. (1978) Estrogenic activity *in vivo* and *in vitro* of some diethylstilbestrol metabolites and analogs. Proc Natl Acad Sci USA 75:468-471.

Korach, K.S., Metzler, M., McLachlan, J.A. (1979) Diethylstilbestrol metabolites and analogs -- New probes for the study of hormone action. J Biol Chem 254:8963-8968.

Korach, K.S., Fox-Davies, C., Quarmby, V.E., Swaisgood, M.G. (1985) Diethylstilbestrol metabolites and analogs – Biochemical probes for differential stimulation of uterine estrogen responses. J Biol Chem 260:1540-15426.

Korach, K.S., Sarver, P., Chae, K., McLachlan, J.D. (1988) Estrogen receptor-binding activity of polychlorinated hydroxybiphenyls: Conformationally restricted structural probes. Mol Pharmacol 33:120-126.

Korenman, S.G. (1969) Comparative binding affinity of estrogens and its relation to estrogenic potency. Steroids:163-177.

Korenman, S.G. (1970) Relation between estrogen inhibitory activity and binding to cytosol of rabbit and human uterus. 87:1119-1123.

Kraichely, D.M., Sun, J., Katzenellenbogen, J.A., Katzenellenbogen, B.S. (2000) Conformational changes and coactivator recruitment by novel ligands for estrogen receptor-α

and estrogen receptor-β: Correlation with biological character and distinct differences among SRC coactivator family members. Endocrinology 141:3534-3545.

Kramer, V.J., Helferich, W.G., Bergman, A., Klasson-Wehler, E., Giesy, J.P. (1997) Hydroxylated polychlorinated biphenyl metabolites are anti-estrogenic in a stably transfected human breast adenocarcinoma (MCF7) cell line. Toxicol Appl Pharmacol 144:363-376.

Kuiper, G.G.J.M., Carlsson, B., Grandien, K., Enmark, E., Haggblad, J., Nillson, S., Gustafsson, J.-A. (1997) Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and ß. Endocrinology 138:863-870.

Kuiper, G.G.J.M., Lemmen, J.G., Carlsson, B., Corton, J.C., Safe, S.H., Saag, P.T., Burg, B., Gustafsson, J.-A. (1998) Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor ß. Endocrinology 139:4252-4263.

Lascombe, I., Beffa, D., Ruegg, U., Tarradellas, J., Wahli, W. (2000) Estrogenic activity assessment of environmental chemicals using *in vitro* assays: Identification of two new estrogenic compounds. Environ Health Perspect 108:621-629.

Laws, S.C., Carey, S.A., Ferrell, J.M., Bodman, G.J., Cooper, R.L. (2000) Estrogenic activity of octylphenol, bisphenol A and methoxychlor in rats. Toxicol Sci 54:154-167.

Laws, S.C., Carey, S.A., Kelce, W.R., Cooper, R.L., Gray, L.E., Jr. (1996) Vinclozolin does not alter progesterone receptor (PR) function *in vivo* despite inhibition of PR binding by its metabolites *in vitro*. Toxicology 112:173-182.

Leibl, H., Spona, J., (1982) Differential stimulation by 17ß -estradiol and synthetic estrogens of progesterone-receptor and of translocation of estrogen-receptor in rat pituitary and uterus. Endocrinology 110:265-271.

Liu, H., Wormke, M., Safe, S.H., Bjeldanes, L.F. (1994) Indolo[3,2-*b*]carbazole: A dietary-derived factor that exhibits both antiestrogenic and estrogenic activity. J Natl Cancer Inst 86:1758-1765.

Matthews, J., Celius, T., Halgren, R., Zacharewski, T. (2000) Differential estrogen receptor binding of estrogenic substances: A species comparison. J Steroid Biochem Mol Biol 74:223-234.

Matthews, J., Zacharewski, T. (2000) Differential binding affinities of PCBs, HO-PCBs, and aroclors with recombinant human, rainbow trout (*Onchorhynkis mykiss*), and green anole (*Anolis carolinensis*) estrogen receptors, using a semi-high throughput competitive binding assay. Toxicol Sci 53:326-339.

Matthews, J.B., Twomey, K., Zacharewski, T.R. (2001) *In vitro* and *in vivo* interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors α and ß. Chem Res Toxicol 14:149-157.

McBlain, W.A. (1987) The levo enantiomer of *o,p'*-DDT inhibits the binding of 17ß-estradiol to the estrogen receptor. Life Sci 40:215-221.

Meyers, M.J., Sun, J., Carlson, K.E., Katzenellenbogen, B.S., Katzenellenbogen, J.A. (1999) Estrogen receptor subtype-selective ligands: Asymmetric synthesis and biological evaluation of *cis*- and *trans*-5,11-Dialkyl-5,6,11,12-tetrahydrochrysenes. J Med Chem 42:2456-2468.

Miodini, P., Fioravanti, L., Di Fronzo, G., Cappelletti, V. (1999) The two phyto-oestrogens genistein and quercetin exert different effects on oestrogen receptor function. Br J Cancer 80:1150-1155.

Morito, K., Hirose, T., Kinjo, J., Hirakawa, T., Okawa, M., Nohara, T., Ogawa, S., Inoue, S., Muramatsu, M., Masamune, Y. (2001). Interaction of phytoestrogens with estrogen receptors α and ß. Biol Pharm Bull 24:351-356.

Nagel, S.C., Saal, F.Sv., Thayer, K.A., Dhar, M.G., Boechler, M., Welshons, W.V. (1997) Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative *in vivo* bioactivity of the xenoestrogens, bisphenol A and octylphenol. Environ Health Perspect 105:70-76.

Nelson, J.A. (1974) Effects of dichlorodiphenyltrichloroethane (DDT) analogs and polychlorinated biphenyl (PCB) mixtures on 17ß-[^{3}H]estradiol binding to rat uterine receptor. Biochem Pharmacol 23(2):447-451.

Nikov, G.N., Eshete, M., Rajnarayanan, R.V., Alworth, W.L. (2001) Interactions of synthetic estrogens with human estrogen receptors. J Endocrinol 170:137-145.

Nikov, G.N., Hopkins, N.E., Boue, S., Alworth, W.L. (2000) Interactions of dietary estrogens with human estrogen receptors and the effect on estrogen receptor-estrogen response element complex formation. Environ Health Perspect 108:867-872.

Olea, N., Pulgar, R., Perez, P., Olea-Serrano, F., Rivas, A., Novillo-Fetrell, A., Pedraza, V., Soto, A.M., Sonnenschein, C. (1996) Estrogenicity of resin-based composites and sealants used in dentistry. Environ Health Perspect 104:298-305.

Palomino, E., Heeg, M.J., Horwitz, J.P., Polin, L., Brooks, S.C. (1994) Skeletal conformations and receptor binding of some 9,11-modified estradiols. J Steroid Biochem Mol Biol 50:75-84.

Parker, G.J., Law, T.L., Lenoch, F.J., Bolger, R.E. (2000) Development of high throughput screening assays using fluorescence polarization: Nuclear receptor-ligand-binding and kinase/phosphatase assays. J Biomol Screen 5:77-88.

Perez, P., Pulgar, R., Olea-Serrano, F., Villalobos, M., Rivas, A., Metzler, M., Pedraza, V., Olea, N. (1998) The estrogenicity of bisphenol A-related diphenylalkanes with various substituents at the central carbon and the hydroxy groups. Environ Health Perspect 106:167-174.

Qian, X., Abul-Hajj, Y.J. (1990) Synthesis and biologic activities of 11ß-substituted estradiol as potential antiestrogens. Steroids 55:238-241.

Ramamoorthy, K., Vyhlidal, C., Wang, F., Chen, I.-C., Safe, S., McDonnell, D.P., Leonard, L.S., Gaido, K.W. (1997b) Additive estrogenic activities of a binary mixture of 2',4',6'-trichloro- and 2',3',4',5' -tetrachloro-4-biphenylol. Toxicol Appl Pharmacol 147:93-100.

Ramamoorthy, K., Wang, F., Chen, I.-C., Norris, J.D., McDonnell, D.P., Leonard, L.S., Gaido, K.W., Bocchinfuso, W.P., Korach, K.S., Safe, S. (1997a) Estrogenic activity of a dieldrin/toxaphene mixture in the mouse uterus, MCF-7 human breast cancer cells, and yeast-based estrogen receptor assays: No apparent synergism. Endocrinology 138:1520-1527.

Rijks, L.J.M., Boer, G.J., Endert, E., de Bruin, K., van den Bos, J.C., van Doremalen, P.A.P.M., Schoonen, W.G.E.J., Janssen, A.G.M., van Royen, E.A. (1996) The stereoisomers of 17α-[^{123}I]iodovinyloestradiol and its 11β-methoxy derivative evaluated for their oestrogen receptor binding in human MCF-7 cells and rat uterus, and their distribution in immature rats. European J Nucl Med 23:295-307.

Routledge, E.J., Parker, J., Odum, J., Ashby, J., Sumpter, J.P. (1998) Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. Toxicol Appl Pharmacol 153:12-19.

Saito, K., Tomigahara, Y., Ohe, N., Isobe, N., Nakatsuka, I., Kaneko, H. (2000) Lack of significant estrogenic or antiestrogenic activity of pyrethroid insecticides in three *in vitro* assays based on classic estrogen receptor-α-mediated mechanisms. Toxicol Sci 57:54-60.

Shelby, M.D., Newbold, R.R., Tully, D.B., Chae, K., Davis, V.L. (1996) Assessing environmental chemicals for estrogenicity using a combination of *in vitro* and *in vivo* assays. Environ Health Perspect 104:1296-1300.

Soto, A.M., Sonnenschein, C., Chung, K.L., Fernandez, M.F., Olea, N., Serrano, F.O. (1995) The E-SCREEN assay as a tool to identify estrogens: An update on estrogenic environmental pollutants. Environ Health Perspect 103(Suppl 7):113-122.

Stoessel, S., Leclercq, G. (1986) Competitive binding assay for estrogen receptor in monolayer culture: Measure of receptor activation potency. J Steroid Biochem Mol Biol 25:677-682.

Sun, J., Meyers, M.J., Fink, B.E., Rajendran, R., Katzenellenbogen, J.A., Katzenellenbogen, B.S. (1999) Novel ligands that function as selective estrogens or antiestrogens for estrogen receptor-α or estrogen receptor-β. Endocrinology 140:800-804.

Vakharia D., Gierthy, J. (1999) Rapid assay for oestrogen receptor binding to PCB metabolites. Toxicol In Vitro 13:275-282

Vakharia, D.D., Gierthy, J.F. (2000) Use of a combined human liver microsome-estrogen receptor binding assay to assess potential estrogen modulating activity of PCB metabolites. Toxicol Lett 14:55-65.

VanderKuur, J.A., Wiese, T., Brooks, S.C. (1993) Influence of estrogen structure on nuclear binding and progesterone receptor induction by the receptor complex. Biochemistry 32:7002-7008.

Waller, C.L., Oprea, T.I., Chae, K., Park, H.-K., Korach, K.S., Laws, S.C., Wiese, T.E., Kelce, W.R., Gray, L.E., Jr. (1996) Ligand-based identification of environmental estrogens. Chem Res Toxicol 9:1240-1248.

Zacharewski, T.R., Meek, M.D., Clemons, J.H., Wu, Z.F., Fielden, M.R., Matthews, J.B. (1998) Examination of the *in vitro* and *in vivo* estrogenic activities of eight commercial phthalate esters. Toxicol Sci 46:282-293.

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Appendix E

Assay Distribution of Substances Tested in *In Vitro* ER Binding Assays

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Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERαdef	GST-mERαdef	GST-rtERdef	hERα	hERα-FP	hERβ	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rERβ	RUC	Total No. of Assays
4,4'-(1,3-Adamantanediyl)diphenol								1								1
2-(1-Adamantyl)-4-methylphenol	41031-50-9							1								1
4-(1-Adamantyl)phenol	29799-07-3							1								1
Alachlor	15972-60-8						1								1	2
Aldosterone	52-39-1														1	1
Aldrin	309-00-2														1	1
Allethrin	584-79-2							1								1
p -(7-Alloxyl)-11-ethyldibenzo-[*b,f*]thiepin-10-yl)phenol	85850-86-8														1	1
p -(3-(Alloxyl)-11-ethyl-6H-dibenzo[*b,f*]thiocin-12-yl)phenol hemihydrate	85850-88-0														1	1
p -(2-(Alloxyl)-6-ethyl-11,12-dihydroxydibenzo[*a,e*]cyclooctene-5-yl)phenol	85850-87-9														1	1
3-(Alloxyl)-10-ethyl-11-(4-hydroxyphenyl)dibenzo[*b,f*]thiepin	85850-85-7														1	1
3-(Alloxyl)-11-ethyl-12-phenyl 6H-dibenzo[*b,f*]thiocin	85850-84-6														1	1
3-(Alloxyl)-10-ethyl-11-phenyldibenzo[*b,f*]thiepin	85850-82-4														1	1
3- (Alloxyl)-10-ethyl-11-phenyldibenz[*b,f*]oxepin	83807-07-2														1	1
3-(Alloxyl)-11-ethyl-12-phenyl 5,6-dihydroxydibenzo[*a,e*]cyclooctene	85850-83-5														1	1
Amaranth	915-67-3														1	1
2-Aminoestratriene-3,17β-diol	107900-30-1										1					1
2-Aminoestratrien-17β-ol	17522-06-4										1					1
4-Aminoestratrien-17β-ol	17522-04-2										1					1
4-Aminoestratriene-3,17β-diol	107900-31-2										1					1
4-Aminophenyl ether	101-80-4														1	1
4-*tert* -Amylphenol	80-46-6						1		1						1	3
3β-Androstanediol	25126-76-5						1							1		2
5α-Androstane-3α,17β-diol	1852-53-5										1	1			1	3
5α-Androstane-3β,17β-diol	571-20-0						1				1	1		1	1	5
5β-Androstane-3α,17β-diol	1851-23-6											1				1
5β-Androstanedione	1229-12-5						1							1		2
5β-Androstane-3,17-dione	5982-99-0						1					1		1		3
5α-Androstane-3α-ol-17-one	53-41-8											1				1
4-Androstenediol	1156-92-9						1							1		2
5-Androstenediol	521-17-5						3		2				1	1		4
4-Androstenedione	63-05-8						1				1	1		1		4

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERαdef	GST-mERαdef	GST-rtERdef	hERα	hERα-FP	hERβ	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rERβ	RUC	Total No. of Assays
Anthracene	120-12-7									1						1
Apigenin	520-36-5						1		2						1	3
Aroclor 1221	11104-28-2														1	1
Aroclor 1254	11097-69-1														1	1
Atrazine	1912-24-9	1	1	1	1	1		1							2	7
Aurin	603-45-2														1	1
Baicalein	491-67-8														1	1
Benomyl	17804-35-2						1									1
Benz[*a*]anthracene	56-55-3			1					1	1						3
Benzeneacetonitrile, a-bis(4-hydroxyphenyl) methylene	66422-14-8									1	1					2
Benzo[*a*]carbazole	239-01-0			1					1							2
Benzo[*c*]carbazole				1					1							2
Benzo[*b*]fluoranthene	205-99-2									1						1
Benzo[*k*]fluoranthene	207-08-9									1						1
Benzo[*a*]fluorene	238-84-6														1	1
Benzo[*b*]fluorene	243-17-4			1					1							2
Benzo[*b*]naptho[2,1-d]thiophene	239-35-0			1					1							2
Benzo[*b*]naptho[2,3-d]thiophene	243-46-9			1					1							2
Benzo[*ghi*]perylene	191-24-2									1						1
Benzo[*c*]phenanthrene	195-19-7			1					1							2
Benzo[*e*]pyrene	192-97-2									1						1
Benzo[*a*]pyrene	50-32-8									1						1
Benzyl alcohol	100-51-6														1	1
4-Benzyloxyphenol	103-16-2														1	1
Benzylparaben	94-18-8														1	1
Biochanin A	491-80-5						1		1						1	3
Bis(*m* -acetoxy)-1,1,2-triphenylbut-1-ene	100808-56-8														1	1
Bis(*p* -acetoxy)-1,1,2-triphenylbut-1-ene	100808-54-6														1	1
Bisdesoxyestradiol	1217-09-0														1	1
1,1-Bis-(4-hydroxyphenyl) ethane	2081/8/5														1	1
4,4-Bis(4-hydroxyphenyl) heptane	7425-79-8														1	1
3,4-Bis(3-hydroxyphenyl)hexane	68266-24-0									1	1					2
3,3-Bis(4-hydroxyphenyl) pentane	3600-64-4														1	1
1,1-Bis(4-hydroxyphenyl) propane	1576-13-2														1	1
2,2-Bis(4-hydroxyphenyl) propanol	142648-65-5														1	1
(2,2-Bis(*p* -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0	1	1	1	1	1	1	1	1			1			3	10
Bisphenol A	80-05-7	1	1	1	1	1	3	2	2	1	1	1	1	1	5	14

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERαdef	GST-mERαdef	GST-rtERdef	hERα	hERα-FP	hERβ	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rERβ	RUC	Total No. of Assays
Bisphenol A bis(chloroformate)	2024-88-6														1	1
Bisphenol A diglycidyl ether	1675-54-3														2	1
Bisphenol A diglycidyl ether dimethacrylate	1565-94-2							1							2	2
Bisphenol A dimethacrylate	3253-39-2												1		2	2
Bisphenol A ethoxylate	68140-85-2														1	1
Bisphenol A ethoxylate diacrylate	64401-02-1														1	1
Bisphenol A glucuronide												1				1
Bisphenol A propoxylate	37353-75-6														1	1
Bisphenol AF	1478-61-1														1	1
Bisphenol B	77-40-7														2	1
Bisphenol C	79-97-0														1	1
Bisphenol C 2	14868-03-2									1	1				1	3
Bisphenol E	6052-84-2														1	1
2,2'-Bisphenol F	2467-02-9														1	1
4,4'-Bisphenol F	620-92-8														2	1
Bisphenol S	80-09-1														1	1
16α-Bromo-17β-estradiol	54982-79-5						2		2							2
1,3-Butanediol, 4-[4-(1,2,3,4-tetrahydro-6-hydroxy-2-phenyl-1-naphthalenyl) phenoxy]-	107144-85-4									1	1					2
1,3-Butanediol, 4-[4-[1,2,3,4-tetrahydro-6-methoxy-2-phenyl1-naphthenyl)phenoxy]-	107163-56-4									1	1					2
Butolame	150748-23-5														1	1
Butyl 4-aminobenzoate	94-25-7														1	1
n -Butylbenzene	104-51-8														1	1
sec -Butylbenzene	135-98-8														1	1
Butyl benzyl phthalate	85-68-7	1	1	1	1	1		2					1		5	8
Butylparaben	94-26-8														2	1
2-*sec* -Butylphenol	89-72-5														1	1
2-*tert* -Butylphenol	88-18-6														1	1
3-*tert* -Butylphenol	585-34-2														1	1
4-*sec* -Butylphenol	99-71-8														1	1
4-*tert* -Butylphenol	98-54-4						1		1						2	3
Butyl phthalyl *n* -butyl glycolate	85-70-1							1								1
Caffeine	58-08-2														1	1
Carbaryl	63-25-2														1	1
Carbofuran	1563-66-2														1	1
Castor oil	8001-79-4														1	1
(±)-Catechin	7295-85-4														1	1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERαdef	GST-mERαdef	GST-rtERdef	hERα	hERα-FP	hERβ	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rERβ	RUC	Total No. of Assays
Chalcone	94-41-7														1	1
Chlordane	57-74-9						1									1
α-Chlordane	5103-71-9														1	1
Chlormequat chloride	999-81-5												1			1
2-Chloro-4-amino-6-isopropylamino-1,3,5-triazine	6190-65-4							1								1
2'-Chloro-4,4'-biphenyldiol	56858-70-9											1				1
2-Chloro-4-biphenylol	23719-22-4											1			1	2
4-Chloro-4'-biphenylol	28034-99-3											2			1	2
2-Chloro-4,6-diamino-*S* -triazine	3397-62-4														1	1
2-Chloro-4-ethylamino-6-amino-1,3,5-triazine	1007-28-9							1								1
2-Chloro-4-ethylamino-6-(1-hydroxyisopropyl)amino-1,3,5-triazine	142179-80-4							1								1
2-Chloro-4-isopropylamino-6-(1-hydroxyisopropylamino)-1,3,5-triazine	142200-36-0							1								1
11β-Chloromethylestradiol	71794-60-0									1	1					2
2-Chloro-4-methylphenol	6640-27-3														1	1
4-Chloro-2-methylphenol	1570-64-5														1	1
4-Chloro-*m* -cresol	59-50-7														1	1
2-Chlorophenol	95-57-8														1	1
4-Chlorophenol	106-48-9														1	1
Chlorotamoxifen	77588-46-6														1	1
Cholesterol	57-88-5														1	1
Chrysene	218-01-9			1					1	1					1	4
Chrysin	480-40-0						1		1						1	3
Cineole	470-82-6														1	1
Cinnamic acid	621-82-9														1	1
cis -Clomiphene	15690-55-8												1			1
trans -Clomiphene	911-45-5						1						1	1		3
Clomiphene citrate	50-41-9														1	1
Colchicine	64-86-8												1			1
Corticosterone	50-22-6						1							1	1	3
Cortisol	50-23-7											1				1
Coumestrol	479-13-0	1	1	1	1	1	3	1	2		1			1	2	11
p -Cumyl phenol	599-64-4														1	1
Cyclofenil diphenol	5189-40-2									1	1					2
Cycloprop[14*R* ,15α]estra-1,3,5(10)-triene-3,17β-diol, 3',15-dihydro-	73860-54-5									1	1					2

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERαdef	GST-mERαdef	GST-rtERdef	hERα	hERα-FP	hERβ	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rERβ	RUC	Total No. of Assays
Cycloprop[14*S* ,15β]estra-1,3,5(10)-triene-3,17β-diol, 3', 15-dihydro-	105455-76-3									1	1					2
Cypermethrin	52315-07-8							1								1
Daidzein	486-66-8						3	1	3						1	4
m,p′ -DDD	4329-12-8														1	1
o,p′ -DDD	53-19-0						2		1						2	3
p,p′ -DDD	72-54-8						2		1						3	3
o,p′ -DDE	3424-82-6	1	1	1	1	1	1		1						2	8
p,p′ -DDE	72-55-9	1	1	1	1	1	1		1				1		3	9
o,p′ -DDT	789-02-6	1	1	1	1	1	2	1	1		1	1	1		5	12
(-)-*o,p′* -DDT	58633-26-4									1					1	2
(+)-*o,p′* -DDT	58633-27-5									1					1	2
p,p′ -DDT	50-29-3	1	1	1	1	1	1	1	1						3	9
Dehydroepiandrosterone	53-43-0	1	1	1	1	1	1							1		7
14-Dehydroestradiol-17β	58699-19-7														1	1
9, 11-Dehydroestradiol											1					1
14 Dehydroestradiol-17β 3-methyl ether	35664-58-7														1	1
14-Dehydroestrone	2119-18-8														1	1
14-Dehydroestrone 3-methyl ether	17550-11-7														1	1
3-Deoxyestradiol	2529-64-8									1	1				1	3
3-Deoxyestrone	53-45-2														1	1
(*R*)-4'-Deoxyindenestrol A	138515-00-1											1				1
(*R*)-5-Deoxyindenestrol A	138515-02-3											1				1
(*rac*)-4'-Deoxyindenestrol A												1				1
(*rac*)-5-Deoxyindenestrol A	138472-84-1											1				1
(*S*)-4'-Deoxyindenestrol A	138514-99-5											1				1
(*S*)-5-Deoxyindenestrol A	138515-01-2											1				1
17-Desoxyestradiol	53-63-4									1	1				3	3
Dexamethasone	50-02-2														1	1
1,3-Diacetoxy-17α-ethinyl-7α-methyl-1,3,5(10)-estratrien-17β-ol															1	1
4,4'-Diaminostilbene dihydrochloride	66635-40-3														1	1
Dibenz[*ah*]anthracene	53-70-3									1						1
Dibenzo-18-crown-6	14187-32-7														1	1
1,3-Dibenzoyloxy-17α-ethinyl-7α-methyl-1,3,5(10)-estratrien-17β-ol															1	1
1,3-Dibenzyltetramethyldisiloxane															1	1
Dibutyl benzyl phthalate		1	1	1	1	1										5
2,6-Di-*tert* -butylphenol	128-39-2														1	1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERαdef	GST-mERαdef	GST-rtERdef	hERα	hERα-FP	hERβ	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rERβ	RUC	Total No. of Assays
Dibutyl phthalate	84-74-2							1					1		3	3
2,4'-Dichlorobiphenyl	34883-43-7														1	1
2,5-Dichlorobiphenyl	34883-39-1						1									1
3,4-Dichlorobiphenyl	2974-92-7						1									1
3,5-Dichlorobiphenyl	34883-41-5						1									1
4,4'-Dichlorobiphenyl	2050-68-2														1	1
2,5-Dichloro-2'-biphenylol	53905-30-9						1									1
2,5-Dichloro-3'-biphenylol	53905-29-6						1									1
2',5'-Dichloro-4-biphenylol	53905-28-5						1					2			1	3
2',6'-Dichloro-4-biphenylol	79881-33-7											1				1
3,4-Dichloro-2'-biphenylol	209613-97-8						1									1
3,4-Dichloro-3'-biphenylol	14962-34-6						1									1
3,4-Dichloro-4'-biphenylol	53890-77-0						1									1
3,5-Dichloro-2'-biphenylol							1									1
3,5-Dichloro-4'-biphenylol							1									1
3,5-Dichloro 2-hydroxy-2-methylbut-3-enanalide	16776-82-1														2	1
2,4-Dichlorophenoxyacetic acid	94-75-7														1	1
2-[[(3,5-Dichlorophenyl)amino]-carbonyl]oxy]-2-methyl 3-butenoic acid	119209-27-7														2	1
Dieldrin	60-57-1						1	1				1			2	4
Dienestrol	84-17-3						1							1	1	3
α-Dienestrol	13029-44-2											1				1
β-Dienestrol	35495-11-5											1				1
1,3-Diethyl-6,4'-dihydroxy-2-phenylindene															1	1
Di 2-ethylhexyl adipate	103-23-1							1								1
Diethylhexyl phthalate	117-81-7							1							2	2
1,3-Diethyl-4-hydroxy-2-phenylindene															1	1
1,3-Diethyl-6-hydroxy 2-phenylindene															1	1
meso-p -(α,β-Diethyl-*p* -methylphenethyl)phenol	267408-76-4														1	1
Diethyl phthalate	84-66-2														1	1
Diethylstilbestrol	56-53-1	1	1	1	1	1	3	4	2	1	2	7	2	1	11	14
3,3'-Diethylstilbestrol	5959-71-7									1	1					2
Diethylstilbestrol dimethyl ether	130-79-0														1	1
Diethylstilbestrol epoxide	6052-82-0											1				1
Diethylstilbestrol-phenanthrene												1				1
5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol (*rac*)							1		1							2
5,11-*trans* -Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							1		1							2

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERαdef	GST-mERαdef	GST-rtERdef	hERα	hERα-FP	hERβ	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rERβ	RUC	Total No. of Assays
(5*R* ,11*R*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							1		1							2
(5*S* ,11*S*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							1		1							2
Dihexyl phthalate	84-75-3														1	1
5,6-Dihydro-8-[2-(dimethylamino)ethoxy]-12-ethyl-11-phenyl-dibenzo[a,e]cyclooctene, hydrate (1:4)	85850-78-8														1	1
Dihydrogenistein	21554-71-2						1		1							2
Dihydroglycitein	94105-88-1								1							1
5α-Dihydrotestosterone	521-18-6	1	1	1	1	1	1	1				1		1	2	10
5β-Dihydrotestosterone	571-22-2														1	1
2,2'-Dihydroxybenzophenone	835-11-0														1	1
2,4-Dihydroxybenzophenone	131-56-6														1	1
4,4'-Dihydroxybenzophenone	611-99-4														1	1
4,4'-Dihydroxybiphenyl	92-88-6						1		1			1				3
Dihydroxydiethylstilbestrol	7507-01-9											1				1
6,4'-Dihydroxyflavone	63046-09-3														1	1
3,3'-Dihydroxyhexestrol	79199-51-2														1	1
2,2'-Dihydroxy-4-methoxybenzophenone	131-53-3														1	1
3-(2,3 Dihydroxypropoxy)-10-ethyl-11-phenyldibenz[*b,f*]oxepin	85850-89-1														1	1
Diisobutyl phthalate	84-69-5														1	1
Diisodecyl phthalate	26761-40-0														1	1
Diisoheptyl phthalate	41451-28-9														1	1
Diisononyl phthalate	28553-12-0														2	1
11β-[2-(N-N-Dimethylamino)ethoxy]estra-1,3,5 (10)-triene-3,17β-diol															1	1
3-[2-(Dimethylamino)ethoxy]-11-ethyl-12-phenyl-6H-dibenzo[*b,f*]thioctin	85850-79-9														1	1
3-[2-(Dimethylamino)ethoxy]-10-ethyl-11-phenyldibenz[*b,f*]oxepin	85850-76-6														1	1
7-[2-(Dimethylamino)ethoxy]-11-ethyl-10-phenyldibenz[*b,f*]thiepin	85850-77-7														1	1
11β-[3-(N-N'-Dimethylamino)propoxy]estra-1,3,5 (10)-triene-3,17β-diol	130043-38-8														1	1
α,α-Dimethyl-β-ethylallenolic acid	15372-37-9														1	1
2,6-Dimethylhexestrol	334707-28-7														1	1
1,6-Dimethylnaphthalene	575-43-9														1	1
Dimethyl phthalate	131-11-3														1	1
α,α-Dimethylstilbestrol	552-80-7												1		1	2
Dimethyl sulfoxide	2206-27-1									1						1
5,11-*trans* -Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							1		1							2

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERαdef	GST-mERαdef	GST-rtERdef	hERα	hERα-FP	hERβ	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rERβ	RUC	Total No. of Assays
(5*R* ,11*R*)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							1		1							2
(5*S* ,11*S*)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol							1		1							2
Di-*n* -octyl phthalate	117-84-0														2	1
Diphenolic acid	126-00-1														1	1
trans, trans -1,4-Diphenyl-1,3-butadiene	886-65-7														1	1
4-[1,2-(Diphenyl-1-butenyl)]phenol acetate	100808-55-7														1	1
2,3-Diphenylindenone-1															1	1
4-[1-(Diphenylmethylene)propyl]phenol acetate	82333-68-4														1	1
1,3-Diphenyltetramethyldisiloxane	56-33-7														1	1
5,11-*trans* -Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol							1		1							2
(5*R* ,11*R*)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol							1		1							2
(5*S* ,11*S*)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol							1		1							2
4-Dodecylphenol	104-43-8														1	1
Doisynoestrol	15372-34-6														1	1
Dopamine	51-61-6														1	1
Droloxifene	82413-20-5									1	1				1	3
Empenthrin	54406-48-3							1								1
α−Endosulfan	959-98-8	1	1	1	1	1	1									6
α,β-Endosulfan	115-29-7						1		1			1	1		2	5
β-Endosulfan	33213-65-9	1	1	1	1	1	1				1					7
16-Epiestriol	547-81-9												1			1
17-Epiestriol	1228-72-4						1		1							2
Epitestosterone	481-30-1														1	1
Equilenin	517-09-9												1			1
Equilin	474-86-2												1			1
Equol	531-95-3						1		1						1	3
Erythro-MEA	20576-52-7												1			1
16α-Estradiol	1090-04-6										1		1			2
17α-Estradiol	57-91-0						2		1		1	1	1	1	2	7
17β-Estradiol	50-28-2	2	1	3	1	2	13	7	10	4	6	12	2	1	19	14
9β-Estradiol											1					1
Estradiol 17-acetate													1			1
17β-Estradiol 3-acetate	4245-41-4												1			1
Estradiol 3-benzoate	50-50-0	1	1	1	1	1										5
Estradiol diacetate	3434-88-6												1			1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERαdef	GST-mERαdef	GST-rtERdef	hERα	hERα-FP	hERβ	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rERβ	RUC	Total No. of Assays
17β-Estradiol 3-methyl ether	1035-77-4												1		1	2
9-Estratetraene-3,17β-diol	791-69-5									1	1					2
Estra-1,3,5(10),6-tetraen-17-one, 3-hydroxy-													1			1
Estra-1,3,5(10)-triene-3,17β-diol, 14,15α-epoxy-	79581-12-7									1	1					2
Estra-1,3,5(10)-triene-3,17β-diol, 14β,15β-epoxy-	79645-49-1									1	1					2
Estra-1,3,5,(10),trien-3,14,17β-triol	16288-09-8									1	1					2
Estratriene-3,6α,17β-triol	1229-24-9														1	1
Estriol	50-27-1	1	1	1	1	1	1			1	3	1	1	1	2	12
Estrone	53-16-7	1	1	1	1	1	1	1		1	3	1	1	1	4	13
Estrone 3-acetate	901-93-9												1			1
Estrone 3-methyl ether	1624-62-0														1	1
Estrone 3-sulfate	481-97-0						1							1		2
17α-Ethinyl estradiol	57-63-6	1	1	1	1	1							1		4	7
17β-Ethinyl estradiol	4717-38-8														1	1
Ethyl cinnamate	103-36-6														1	1
3-Ethyl-6,4'-dihydroxy-2-phenylindene															1	1
2-Ethylhexyl paraben	5153-25-3														1	1
3-Ethyl-4'-hydroxy 2-phenylindenone-1															1	1
3-Ethyl-6-hydroxy 2-phenylindenone-1															1	1
4-Ethyl-7-hydroxy-3-(4-methoxyphenyl)-2*H* -1-benzopyran-2-one	5219-17-0														1	1
3-[(10-Ethyl-11-*p* -hydroxyphenyl)dibenzo-[*b,f*]oxepin-3-yl)oxy]-1,2-propanediol, hydrate (4:1)	85850-93-7														1	1
3-[(10-Ethyl-11-*p* -hydroxyphenyl)dibenzo-[*b,f*]thiepin-3-yl)oxy]-1,2-propanediol	85850-94-8														1	1
3-[(11-Ethyl-12-(*p* -hydroxyphenyl)-6-*H* -dibenzo-[*b,f*]thiocin-3-yl)oxy]-, hemihydrate 1,2-propanediol	85864-54-6														1	1
3-[(6-Ethyl-5-(*p* -hydroxyphenyl)-11,12-dihydrodibenzo[*a,e*]cycloocten-2-yl)oxy]-1,2-propanediol	85850-95-9														1	1
3-Ethyl-4'-hydroxy-2-phenylindene															1	1
3-Ethyl-6-hydroxy 2-phenylindene															1	1
3-Ethyl-4-(*p* -methoxyphenyl)-2-methyl-3-cyclohexene-1-carboxylic acid	1755-52-8												1			1
Ethyl paraben	120-47-8														1	1
2-Ethylphenol	90-00-6														1	1
3-Ethylphenol	620-17-7														1	1
4-Ethylphenol	123-07-9														1	1
3-[(10-Ethyl-11-phenyldibenzo[*b,f*]thiepin-3-yl)oxy]-1,2-propanediol, complexed with isopropyl alcohol 2:1	85850-90-4														1	1
3-[(11-Ethyl-12-phenyl-6*H* -dibenzo[*b,f*]thioctin-3-yl)oxy]-1,2-propanediol, hydrate (4:1)	85850-92-6														1	1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERαdef	GST-mERαdef	GST-rtERdef	hERα	hERα-FP	hERβ	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rERβ	RUC	Total No. of Assays
3-[6-Ethyl-5-phenyl-11,12-dihydrodibenzo[*a,e*]cycloocten-2-yl)oxy]-1,2-propanediol	85850-91-5														1	1
Eugenol	97-53-0														1	1
Fenvalerate	51630-58-1							1								1
Fisetin	528-48-3														1	1
Flavanone	17002-31-2														1	1
Flavone	525-82-6						1		1						1	3
Fluoranthene	206-44-0									1						1
Fluorene	86-73-7									1						1
4-Fluoroestratrien-17β-ol	96607-54-4										1					1
2-Fluoroestratrien-17β-ol	101772-22-9										1					1
2-(2-Fluorophenyl)-3-phenyl-6-hydroxyindene															1	1
Fluorotamoxifen	73617-96-6														1	1
Folic acid	59-30-3														1	1
Formononetin	485-72-3						1		1						1	3
Furfural	98-01-1														1	1
Genistein	446-72-0	1	1	1	1	1	4	1	3		2			1	2	11
Genistin	529-59-9								1						1	2
Glyceollin	66241-09-6							1								1
Glycitein	40957-83-3						1		1							2
Glycitin									1							1
Heptachlor	76-44-8														1	1
2,2',3,3',4',5',6'-Heptachlorobiphenyl	52663-70-4	1		1		1										3
2,2',3,3',4,5,6,-Heptachlorobiphenyl	68194-16-1	1		1		1										3
2,2',3,3',5,5',6-Heptachlorobiphenyl	52663-64-6	1		1		1										3
2,2',3,4,4',5',6-Heptachlorobiphenyl	52663-69-1	1		1		1										3
2,2',3,4,4',6,6'-Heptachlorobiphenyl	74472-48-3	1		1		1										3
2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0	1		1		1										3
2,2',3,4',5,6,6'-Heptachlorobiphenyl	74487-85-7	1		1		1										3
2,3,3',4,4',5,6-Heptachlorobiphenyl	41411-64-7	1		1		1										3
2,3,3',4',5,5',6-Heptachlorobiphenyl	69782-91-8	1		1		1										3
2,2',3,3',4',5,5'-Heptachloro-4-biphenylol	158076-64-3	1		1		1	1		1							5
2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8	1		1		1	1		1							5
2,2',3,4',5,5',6-Heptachloro-4-biphenylol	158076-68-7						1		1							2
Heptanal	111-71-7														1	1
4-(Heptyloxy)phenol	13037-86-0														1	1
Heptyl 4-paraben	1085-12-7														1	1
Hesperetin	520-33-2														1	1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERαdef	GST-mERαdef	GST-rtERdef	hERα	hERα-FP	hERβ	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rERβ	RUC	Total No. of Assays
Hexachlorobenzene	118-74-1														1	1
2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3	1		1		1										3
2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2	1		1		1										3
2,2',3,4,5,6'-Hexachlorobiphenyl	68194-15-0	1		1		1										3
2,2',3,5,5',6-Hexachlorobiphenyl	52663-63-5	1		1		1										3
2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	1		1		1										3
2,2',4,4',6,6'-Hexachlorobiphenyl	33979-03-2						1					1				2
2,2',3,4',5',6-Hexachlorobiphenyl	38380-04-0	1		1		1										3
2,3,3',4,4',6-Hexachlorobiphenyl	74472-42-7	1		1		1										3
2,3',4,4',5',6-Hexachlorobiphenyl	59291-65-5	1		1		1										3
3,3',4,4',5,5'-Hexachlorobiphenyl	32774-16-6	1		1		1										3
2,2',3,3',4',5-Hexachloro-4-biphenylol	158076-62-1	1		1		1	1		1							5
2,2',3,4',5,5'-Hexachloro-4-biphenylol	145413-90-7	1		1		1	1		1							5
2',3,3',4',5,5'-Hexachloro-4-biphenylol	158076-63-2										1					1
n -Hexanol	111-27-3														1	1
Hexestrol	84-16-2						1			1	1		1	1	2	6
DL -Hexestrol	5776-72-7														1	1
Hexestrol monomethyl ether	13026-26-1														1	1
3-Hydroxybenzo[*b*]naphtho[2,1-d]thiophene				1					1							2
2-Hydroxybenzo[*c*]phenanthrene	22717-94-8			1					1							2
3-Hydroxybenzo[*b*]phenanthro[2,3-d]thiophene				1					1							2
4'-Hydroxychalcone	2657-25-2														1	1
4-Hydroxychalcone	20426-12-4														1	1
4'-Hydroxychalcone (*cis* - and *trans* -)	38239-52-0														1	1
2-Hydroxychrysene	65945-06-4			1					1							2
4'-Hydroxy-2,3-diphenylindenone-1															1	1
6'-Hydroxy-2,3-diphenylindenone-1															1	1
11β-Hydroxyestradiol	5444-22-4									1	2		1		1	4
11α-Hydroxyestradiol	1464-61-5										1					1
14β-Hydroxyestradiol	60183-66-6									1	1					2
2-Hydroxyestradiol	362-05-0						1							1		2
4-Hydroxyestradiol	5976-61-4						1							1		2
2-Hydroxyestratrien-17β-ol	2259-89-4										1					1
4-Hydroxyestratrien-17β-ol	17592-89-1										1					1
3-Hydroxyestra-1,3,5(10)-trien-16-one	3601-97-6														1	1
2-Hydroxyestrone	362-06-1						1		1							2
2-Hydroxyethyl methacrylate	868-77-9							1								1
3'-Hydroxyflavanone	92496-65-6														1	1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERαdef	GST-mERαdef	GST-rtERdef	hERα	hERα-FP	hERβ	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rERβ	RUC	Total No. of Assays
4'-Hydroxyflavanone	135413-27-3														1	1
6-Hydroxyflavanone	4250-77-5														1	1
7-Hydroxyflavanone	6515-36-2														1	1
6-Hydroxyflavone	6665-83-4														1	1
7-Hydroxyflavone	6665-86-7														1	1
Hydroxyflutamide	52806-53-8														2	1
6-Hydroxy-2'-methoxyflavone	61546-59-6														1	1
2-Hydroxy-4-methoxybenzophenone	131-57-7														1	1
2-Hydroxy-5-methylchrysene				1					1							2
8-Hydroxy-5-methylchrysene				1					1							2
16β-Hydroxy-16-methyl-17β-estradiol 3-methyl ether	3434-79-5														1	1
4-Hydroxytamoxifen	68047-06-3	1	1	1	1	1	3	3	2	1	1	1		1	1	13
6-Hydroxytetralin	1125-78-6														1	1
ICI 164384	98007-99-9	1	1	1	1	1	2		1					1	1	9
ICI 182780	129453-61-8						1		1				1		2	4
Imiprothrin	72963-72-5							1								1
Indanestrol	71855-45-3											2				1
(*S*)-Indenestrol B												1				1
Indanyldiethylstilbestrol												1				1
Indenestrol A	24643-97-8											3				1
(*R*)-Indenestrol A	115217-03-3											1				1
(*S*)-Indenestrol A	115217-04-4											1				1
Indenestrol B	38028-27-2											2				1
(*R*)-Indenestrol B	115217-06-6											1				1
(*rac*)-Indenestrol B	133830-97-4											1				1
Indeno[1,2,3-*cd*]pyrene	193-39-5									1						1
Indole[3,2-*b*]carbazole															1	1
16α-Iodoestradiol	71765-94-1										1					1
(*E*)-17α-Iodovinylestradiol	82123-96-4										1				1	2
(Z)-17α-Iodovinylestradiol	177159-09-0										1				1	2
Ipriflavone	35212-22-7						1		1							2
Isoeugenol	97-54-1														1	1
Kaempferol	520-18-3						1		2						1	3
Kepone	143-50-0	1	1	1	1	1	1	1	1			1			3	10
11-Keto-9β-estradiol											1					1
16-Ketoestradiol	566-75-6						1		1		1		1			4
6-Ketoestradiol	571-92-6									1	1					2
Lindane	58-89-9														2	1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERαdef	GST-mERαdef	GST-rtERdef	hERα	hERα-FP	hERβ	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rERβ	RUC	Total No. of Assays
Melatonin	73-31-4														1	1
MER-25	67-98-1											1			1	
Mestilbol	18839-90-2														1	1
Mestranol	72-33-3												1		2	2
Methoxychlor (*p,p′* -)	72-43-5	1	1	1	1	1	2	1	1		1	1	1	1	5	13
Methoxychlor olefin	2132-70-9														1	1
3-Methoxyestriol	1474-53-9													1	1	
E -11β-Methoxy-17α-iodovinylestradiol	90857-55-9										1				1	2
(*Z*)-11β-Methoxy-17α-iodovinylestradiol	177159-11-4										1				1	2
3-Methoxy-10-methyl-11-phenyldibenzo[*b,f*]thiepin															1	1
Methoxytamoxifen															1	1
7α-Methyl-14-Dehydroestradiol-17β	88598-62-3														1	1
9α-Methyl-14-Dehydroestradiol-17β	88598-63-4														1	1
11β-Methyl-14-dehydroestradiol-17β 3-methyl ether	88598-65-6														1	1
7α-Methyl-14-dehydroestradiol-17β 3-methyl ether	35644-59-8														1	1
9α-Methyl-14-dehydroestradiol-17β 3-methyl ether	88598-64-5														1	1
7α-Methyl-14-dehydroestrone	88958-66-7														1	1
9α-Methyl-14-dehydroestrone	88598-67-8														1	1
11β-Methyl-14-dehydroestrone 3-methyl ether	88598-69-0														1	1
7α-Methyl-14-dehydroestrone 3-methyl ether	35644-57-6														1	1
9α-Methyl-14-dehydroestrone 3-methyl ether															1	1
4,4'-Methylenebis(*N,N* -dimethylbenzeneamine)	101-61-1														1	1
4,4'-Methylenedianiline	101-77-9														1	1
11β-Methylestradiol-17β	23637-93-6									1	1				1	3
7α−Methylestradiol-17β	10448-97-2														1	1
9α-Methylestradiol-17β	66463-44-3														1	1
11β-Methylestradiol-17β 3-methyl ether	18046-75-8														1	1
7α−Methylestradiol-17β 3-methyl ether	15506-01-1														1	1
9α-Methylestradiol-17β 3-methyl ether	51242-32-1														1	1
11β-Methylestrone	13667-06-6														1	1
7α-Methylestrone	10448-96-1														1	1
9α-Methylestrone	71563-77-4														1	1
11β-Methylestrone 3-methyl ether	13667-04-4														1	1
7α-Methylestrone 3-methyl ether	10449-00-0														1	1
9α-Methylestrone 3-methyl ether	31266-41-8														1	1
1-Methyl-3-ethyl-6,4'-dihydroxy-2-phenylindene															1	1
1-Methyl-6-hydroxy-2,3-diphenylindene															1	1
Methyl methacrylate	80-62-6							1								1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERαdef	GST-mERαdef	GST-rtERdef	hERα	hERα-FP	hERβ	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rERβ	RUC	Total No. of Assays
Methyl paraben	99-76-3														1	1
2-(2-Methylphenyl)-3-phenyl-6-hydroxyindene															1	1
Methyltamoxifen	73617-95-5														1	1
Methoxychlor (*p,p′ + o,p′* -)	30667-99-3														1	1
Metolachlor	51218-45-2														1	1
Mirex	2385-85-5														1	1
Monohydroxytamoxifen	68392-35-8														1	1
Mono-*m* -acetoxy-1,1,2-triphenylbut-1-ene	82333-69-5														1	1
Monohydroxymethoxychlor	28463-03-8														1	1
Monohydroxymethoxychlor olefin	75938-34-0														1	1
Morin	480-16-0														1	1
Moxestrol	34816-55-2						1					1		1	1	4
Myricetin	529-44-2														1	1
Nafoxidine	1845-11-0						1			1	1			1	1	5
Naringenin	480-41-1	1	1	1	1	1	1		2						1	8
Naringin	10236-47-2														1	1
Nerolidol	7212-44-4														1	1
2-Nitroestratriene-3,17β-diol	6298-51-7										1					1
4-Nitroestratriene-3,17β-diol	6936-94-3										1					1
2-Nitroestratrien-3-ol-17-one	5976-73-8										1					1
4-Nitroestratrien-3-ol-17-one	5976-74-9										1					1
Nitromifene	10448-84-7												1			1
cis -Nonachlor	5103-73-1						1									1
trans -Nonachlor	39765-80-5						1									1
Nonylbenzene	1081-77-2														1	1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERαdef	GST-mERαdef	GST-rtERdef	hERα	hERα-FP	hERβ	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rERβ	RUC	Total No. of Assays
p -Nonylphenol	104-40-5							2		1	1				10	4
n -Nonylphenol	25154-52-3						2		2			1	1			4
Nonylphenol dodecylethoxylate													1			1
Nordihydroguariaretic acid	500-38-9														1	1
Norethindrone	68-22-4						1							1		2
Norethynodrel	68-23-5						1							1	2	3
19-Nortestosterone	434-22-0						1					1		1		3
2,2'3,3',4,4',5,5'-Octachlorobiphenyl	35694-08-7	1		1		1										3
1,8-Octanediol	629-41-4														1	1
4-*n* -Octylphenol	1806-26-4						1		1	1	1		1		1	6
4-*tert* -Octylphenol	140-66-9	1	1	1	1	1	1	1	1						3	9
2,2',3,3',6-Pentachlorobiphenyl	52663-60-2	1		1		1										3
2,2',3,4,5'-Pentachlorobiphenyl	38380-02-8	1		1		1										3
2,2',3,4',6-Pentachlorobiphenyl	68194-05-8	1		1		1										3
2,2',3,5',6-Pentachlorobiphenyl	38379-99-6	1		1		1										3
2,2',4,4',5-Pentachlorobiphenyl	38380-01-7	1		1		1										3
2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2	1		1		1										3
2,2',4,6,6'-Pentachlorobiphenyl	56558-16-8	1		1		1						1				4
2,3,3',5,6,-Pentachlorobiphenyl	74472-36-9	1		1		1										3
2,3,4,4',6,-Pentachlorobiphenyl	74472-38-1	1		1		1										3
3,3',4,4',5,-Pentachlorobiphenyl	57465-28-8	1		1		1										3
2,2',3',4',5'-Pentachloro-4-biphenylol	150304-12-4						1		1			1			1	4
2,2',3',4',6'-Pentachloro-4-biphenylol	150304-10-2						1		1			1			1	4
2,2',3',5',6'-Pentachloro-4-biphenylol	150304-11-3						1		1			1			1	4
2,2',4,6,6'-Pentachloro-4-biphenylol												1				1
2',3,3',4,4'-Pentachloro-2-biphenylol	150975-80-7										1					1
2,3,3',4',5-Pentachloro-4-biphenylol	152969-11-4	1		1		1	1		1		1					6
2',3,3',4',5'-Pentachloro-4-biphenylol	149589-55-9						1		1			1			1	4
2',3,3',4',5-Pentachloro-4-biphenylol	192190-09-3						1		1			1			1	4
2',3,3',4',6'-Pentachloro-4-biphenylol	192190-10-6						1		1			1			1	4
2',3,3',5',6'-Pentachloro-4-biphenylol	189578-02-7						1		1			1			1	4
2,3',4,4',5-Pentachloro-3-biphenylol	170946-11-9										1					1
2',3',4,4',5-Pentachloro-3-biphenylol	150975-81-8										1					1
2',3,4',5,5'-Pentachloro-4-biphenylol	149589-56-0										1					1
2,2',4,6,6'-Pentachloro-4-biphenylol												1				1
3,3',4',5,5'-Pentachloro-4-biphenylol	130689-92-8										1					1
Pentolame	150748-24-6														1	1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERαdef	GST-mERαdef	GST-rtERdef	hERα	hERα-FP	hERβ	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rERβ	RUC	Total No. of Assays
Permethrin	52645-53-1							1								1
Phenanthrene	85-01-8									1						1
4-Phenethylphenol	6335-83-7														1	1
Phenol, 4,4'-[1,2-bis(methylene)-1,2-ethanediyl]bis	107144-81-0									1	1					2
Phenol, 3-[2-dimethylamino-ethoxy]-10-ethyl-4-hydroxy-phenyl dibenzo[*b,f*]oxepin	85850-80-2									1	1				1	3
Phenol, 4-[7-[2 dimethylamino)ethoxy]-11-ethyldibenzo[*b,f*]thiepin-10-yl)	85850-74-4									1	1				1	3
Phenol, 4-[3-(2 dimethylamino)ethoxy]-11-ethyldibenzo[*b,f*]thioctin-12-yl)-, hydrate (4:1)	85850-81-3									1	1				1	3
Phenol, 4-[2-(2 dimethylamino)ethoxy]-6-ethyl-11,12-dihydrodibenzo[*a,e*]cycloocten-5-yl]	85850-75-5									1	1				1	3
Phenol, 4-[1-[4-[2-(dimethylamino)ethoxy] phenyl]-2-phenyl-1-butenyl]-3-methyl-, (*E*)-	96474-35-0									1	1					2
Phenol, 4-(1, 2-diphenyl-1-butenyl)-	69967-79-9									1	1					2
Phenol, 4,(1*Z*) 1-2-diphenyl-1-butenyl)-	69967-80-2									1	1					2
Phenol, 4,4'-[2-phenyl-1-butenylidene)bis	91221-46-4									1	1					2
Phenolphthalein	77-09-8														1	1
Phenolphthalin	81-90-3														1	1
Phenol Red	143-74-8														1	1
δ-Phenothrin	26002-80-2							1								1
2-Phenyl-3-(2-fluoro-4-hydroxyphenyl)-6-hydroxyindene															1	1
2-Phenyl-3-(2-fluorophenyl)-6-hydroxyindene															1	1
3-Phenyl-4'-hydroxy-2-phenylindene															2	1
3-Phenyl-6-hydroxy-2-phenylindene															1	1
2-Phenyl-3-(2-methylphenyl)-6-hydroxyindene															1	1
2-Phenyl-3-(4-methylphenyl)-6-hydroxyindene															1	1
2-Phenylphenol	90-43-7														1	1
3-Phenylphenol	580-51-8														1	1
4-Phenylphenol	92-69-3											1			1	2
Phloretin	60-82-2						1		1						1	3
Prallethrin	23031-36-9							1								1
Progesterone	57-83-0						2		1			1		1	3	5
Prolame	99876-41-2														1	1
Promegestone	34184-77-5											1			2	2
Prometon	1610-18-0														1	1
Propazine	139-40-2							1								1
Propyl paraben	94-13-3														1	1
Propylpyrazoletriol							1		1							2

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERαdef	GST-mERαdef	GST-rtERdef	hERα	hERα-FP	hERβ	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rERβ	RUC	Total No. of Assays
Prunetin	552-59-0														1	1
Pseudodiethylstilbestrol	39011-86-4											2				1
Pyrene	129-00-0									1						1
Pyrrolidine, 1-[2-[4-[1-(4-methoxyphenyl)-2-nitro-2-phenylethenyl]phenoxy]ethyl]-, (*E*)	77413-87-7									1	1					2
Quercetin	117-39-5	1	1	1	1	1	1		1		1				1	9
6-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-	107144-82-1									1	1					2
7-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-	107144-83-2									1	1					2
Raloxifene	84449-90-1						2		2							2
Raloxifene hydrochloride	82640-04-8						1									1
Resveratrol	501-36-0														1	1
Rutin	153-18-4														1	1
Simazine	122-34-9	1	1	1	1	1		1							1	7
β-Sitosterol	83-46-5	1	1	1	1	1	1							1	1	8
4,4'-Stilbenediol	659-22-3														1	1
4-Stilbenol	3839-46-1														1	1
Suberic acid	505-48-6														1	1
Tamoxifen	10540-29-1	1	1	1	1	1	3	2	2	1	1	1	1	1	4	14
Tamoxifen citrate	54965-24-1														1	1
Taxifolin	480-18-2														1	1
Testosterone	58-22-0						2	1	1			1	1	1	2	7
2,2',3,3'-Tetrachlorobiphenyl	3844-93-8	1		1		1										3
2,2',3,4,-Tetrachlorobiphenyl	52663-59-9	1		1		1										3
2,2',3,6-Tetrachlorobiphenyl	41464-47-5	1		1		1										3
2,2',4,4'-Tetrachlorobiphenyl	2437-79-8	1		1		1									1	4
2,2',4,5'-Tetrachlorobiphenyl	41464-40-8	1		1		1										3
2,2',4,6'-Tetrachlorobiphenyl	68194-04-7	1		1		1										3
2,2',5,5'-Tetrachlorobiphenyl	35693-99-3						1									1
2,2',6,6'-Tetrachlorobiphenyl	15968-05-5	1		1		1	2		1							5
2,3,3',5'-Tetrachlorobiphenyl	41464-49-7	1		1		1										3
2,3,4,4'-Tetrachlorobiphenyl	33025-41-1	1		1		1										3
2,3',4',5-Tetrachlorobiphenyl	32598-11-1	1		1		1										3
2,3',4,5'-Tetrachlorobiphenyl	73575-52-7	1		1		1										3
2,4,4',5-Tetrachlorobiphenyl	32690-93-0	1		1		1										3
2,6,2',6'-Tetrachlorobiphenyl	15968-05-5						1									1
3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	1		1		1	1								1	5

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERαdef	GST-mERαdef	GST-rtERdef	hERα	hERα-FP	hERβ	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rERβ	RUC	Total No. of Assays
3,3',4,5-Tetrachlorobiphenyl	70362-49-1	1		1		1										3
3,3',5,5'-Tetrachloro-4,4'-biphenyldiol	13049-13-3										1	2			1	3
2,2',4',6'-Tetrachloro-4-biphenylol	150304-08-8						1		1			1			1	4
2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8	1		1		1	3		2							5
2',3',4',5'-Tetrachloro-3-biphenylol	67651-37-0										1					1
2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	1		1		1	1		1		1	3			1	8
2',3,4',6'-Tetrachloro-4-biphenylol	189578-00-5						1		1			1			1	4
2',3',5',6'-Tetrachloro-4,4'-biphenyldiol	100702-98-5											1				1
2,3,7,8,-Tetrachlorodibenzo-*p* -dioxin	1746-01-6									1						1
Tetrahydrochrysene	104460-72-2					1		1							2	
(*rac*)-Tetrahydrochrysene							1		1							2
(*R,R*)-Tetrahydrochrysene							1		1							2
(*S,S*)-Tetrahydrochrysene							1		1							2
2,2',4,4'-Tetrahydroxybenzil	5394-98-9														1	1
Tetramethylhexestrol	74385-27-6									1	1					2
Thalidomide	50-35-1														1	1
Toremifene citrate	89778-27-8														1	1
Tosyl Nonylphenol (mixed branched isomers)															1	1
Toxaphene	8001-35-2						1				1	1				3
Triaryl-pyrazole							1		1							2
2,2',5-Trichlorobiphenyl	37680-65-2	1		1		1										3
2,4,6-Trichlorobiphenyl	35693-92-6						1									1
3,3',4-Trichloro-4-biphenylol	124882-64-0										1					1
2',4',6'-Trichloro-4-biphenylol	14962-28-8						2		1		1	3				4
3,4',5-Trichloro-4-biphenylol	4400-06-0											1				1
2,4,5-Trichlorophenoxyacetic acid	93-76-5														1	1
Triethylamine, 2-[*p* -[6-methoxy-2-phenyl-3-inden-3-yl] phenoxy]hydrochloride	64-96-0												1			1
Triethylene glycol dimethacrylate	109-16-0							1								1
4,2',4'-Trihydroxychalcone	961-29-5														1	1
3,6,4'-Trihydroxyflavone	253195-19-6														1	1
6,7,4'-Trihydroxyisoflavone	17817-31-1														1	1
7,3',4'-Trihydroxyisoflavone	485-63-2														1	1
1,1,2-Triphenylbut-1-ene	63019-13-6														1	1
Triphenylethylene	58-72-0														1	1
Triphenyl phosphate	115-86-6														1	1

Assay Distribution of Substances Tested

Substance	CASRN	GST-aERdef	GST-cERdef	GST-hERαdef	GST-mERαdef	GST-rtERdef	hERα	hERα-FP	hERβ	MCF-7 cells	MCF-7 cytosol	MUC	RBC	rERβ	RUC	Total No. of Assays
Tris-4-(chlorophenyl)methane	27575-78-6									1						1
Tris-4-(chlorophenyl)methanol	30100-80-8									1						1
Vanillin	121-33-5														1	1
Vinclozolin	50471-44-8														2	1
α-Zearalanol	26538-44-3														1	1
β-Zearalanol	42422-68-4						1							1	1	3
Zearalanone	5975-78-0														1	1
α-Zearalenol	36455-72-8	1	1	1	1	1									1	6
β-Zearalenol	71030-11-0	1	1	1	1	1									1	6
Zearalenone	17924-92-4	1	1	1	1	1	2	1	2		1				1	10
	TOTALS	85	34	99	34	86	133	48	100	66	94	75	44	38	376	

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Appendix F
Median/Single RBA Values for Substances Tested in Two or More *In Vitro* ER Binding Assays

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Median/Single RBA Values for Sustances Tested in Two or More In Vitro ER Binding Assays

Substance	CASRN	RUC		hERα		hERα-FP		hERβ		rERβ		GST-aERdef		GST-cERdef	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
Alachlor	15972-60-8	neg (100)	(1)	neg (50)	(1)										
4-*tert* -Amylphenol	80-46-6	0.0005	(1)	neg (10)	(1)			neg (10)	(1)						
3ß-Androstanediol	25126-76-5			3	(1)					7	(1)				
5α-Androstane-3α,17ß-diol	1852-53-5	0.002	(1)												
5α-Androstane-3β,17β-diol	571-20-0	0.12	(1)	0.07	(1)					0.3	(1)				
5ß-Androstane-3,17-dione	5982-99-0			neg (100)	(1)					neg (100)	(1)				
5ß-Androstanedione	1229-12-5			neg (100)	(1)					neg (100)	(1)				
4-Androstenediol	1156-92-9			0.5	(1)					0.6	(1)				
5-Androstenediol	521-17-5			3.9	(3)			14.1	(2)	17	(1)				
4-Androstenedione	63-05-8			neg (100)	(1)					neg (100)	(1)				
Apigenin	520-36-5	0.028	(1)	0.3	(1)			4	(2)						
Atrazine	1912-24-9	0.0003	(1/2)			neg (2000)	(1)					neg (100)	(1)	neg (100)	(1)
Benz[*a*]anthracene	56-55-3							neg (10)	(1)						
Benzeneacetonitrile, α-bis(4-hydroxyphenyl)methylene	66422-14-8														
Benzo[*a*]carbazole	239-01-0							neg (10)	(1)						
Benzo [*c*]carbazole								neg (10)	(1)						
Benzo[*b*]fluorene	243-17-4							neg (10)	(1)						
Benzo[*b*]naptho[2,1-d]thiophene	239-35-0							neg (10)	(1)						
Benzo[*b*]naptho[2,3-d]thiophene	243-46-9							neg (10)	(1)						
Benzo[*c*]phenanthrene	195-19-7							neg (10)	(1)						
Biochanin A	491-80-5	0.0043	(1)	neg (10)	(1)			neg (10)	(1)						
3,4-Bis(3-hydroxyphenyl)hexane	68266-24-0														
2,2-Bis(*p* -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0	0.754	(3)	0.4	(1)	1.7	(1)	2	(1)			4.80	(1)	4.8	(1)
Bisphenol A	80-05-7	0.056	(5)	0.01	(3)	0.25	(2)	0.0365	(2)	0.33	(1)	0.13	(1)	0.044	(1)
Bisphenol A diglycidyl ether dimethacrylate	1565-94-2	neg (200)	(2)			neg (5000)	(1)								
Bisphenol A dimethacrylate	3253-39-2	0.017	(2)												
Bisphenol C 2	14868-03-2	2.64	(1)												
16α-Bromo-17ß-estradiol	54982-79-5			65	(2)			13.4	(2)						
1,3-Butanediol, 4-[4-(1,2,3,4-tetrahydro-6-hydroxy-2-phenyl-1-naphthalenyl) phenoxy]-	107144-85-4														
1,3-Butanediol, 4-[4-[1,2,3,4-tetrahydro-6-methoxy-2-phenyl 1-naphthenyl) phenoxy]-	107163-56-4														
Butyl benzyl phthalate	85-68-7	0.0017	(2/5)			0.0061	(2)					neg (100)	(1)	neg (100)	(1)
4-*tert* -Butylphenol	98-54-4	0.0009	(2)	neg (10)	(1)			neg (10)	(1)						
2-Chloro-4-biphenylol	23719-22-4	0.002	(1)												
4-Chloro-4'-biphenylol	28034-99-3	0.007	(1)												

Median/Single RBA Values for Sustances Tested in Two or More In Vitro ER Binding Assays

Substance	CASRN	GST-hERαdef		GST-mERαdef		GST-rtERdef		MCF-7 cells		MCF-7 cytosol		MUC		RBC	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
Alachlor	15972-60-8														
4-*tert* -Amylphenol	80-46-6														
3ß-Androstanediol	25126-76-5														
5α-Androstane-3α,17ß-diol	1852-53-5									neg (0.1)	(1)	neg (10)	(1)		
5α-Androstane-3β,17β-diol	571-20-0									0.005	(1)	0.5	(1)		
5ß-Androstane-3,17-dione	5982-99-0											neg (10)	(1)		
5ß-Androstanedione	1229-12-5														
4-Androstenediol	1156-92-9														
5-Androstenediol	521-17-5													1	(1)
4-Androstenedione	63-05-8									0.007	(1)	neg (10)	(1)		
Apigenin	520-36-5														
Atrazine	1912-24-9	neg (100)	(1)	neg (100)	(1)	neg (100)	(1)								
Benz[*a*]anthracene	56-55-3	neg (10)	(1)					33	(1)						
Benzeneacetonitrile, α-bis(4-hydroxyphenyl)methylene	66422-14-8							8.5	(1)	100	(1)				
Benzo[*a*]carbazole	239-01-0	neg (10)	(1)												
Benzo [*c*]carbazole		neg (10)	(1)												
Benzo[*b*]fluorene	243-17-4	neg (10)	(1)												
Benzo[*b*]naptho[2,1-d]thiophene	239-35-0	neg (10)	(1)												
Benzo[*b*]naptho[2,3-d]thiophene	243-46-9	neg (10)	(1)												
Benzo[*c*]phenanthrene	195-19-7	neg (10)	(1)												
Biochanin A	491-80-5														
3,4-Bis(3-hydroxyphenyl)hexane	68266-24-0							20	(1)	10	(1)				
2,2-Bis(*p* -hydroxyphenyl)-1,1,1 trichlorethane	2971-36-0	1.2	(1)	1.2	(1)	14	(1)					1.2	(1)		
Bisphenol A	80-05-7	0.008	(1)	0.0086	(1)	0.21	(1)	0.006	(1)	1	(1)	0.0115	(1)	0.0013	(1)
Bisphenol A diglycidyl ether dimethacrylate	1565-94-2														
Bisphenol A dimethacrylate	3253-39-2													0.00047	(1)
Bisphenol C 2	14868-03-2							0.3	(1)	2	(1)				
16α-Bromo-17ß-estradiol	54982-79-5														
1,3-Butanediol, 4-[4-(1,2,3,4-tetrahydro-6-hydroxy-2-phenyl-1-naphthalenyl) phenoxy]-	107144-85-4							7	(1)	30	(1)				
1,3-Butanediol, 4-[4-[1,2,3,4-tetrahydro-6-methoxy-2-phenyl 1-naphthenyl) phenoxy]-	107163-56-4							0.06	(1)	0.1	(1)				
Butyl benzyl phthalate	85-68-7	neg (100)	(1)	neg (100)	(1)	neg (100)	(1)							neg (100)	(1)
4-*tert* -Butylphenol	98-54-4														
2-Chloro-4-biphenylol	23719-22-4											0.04	(1)		
4-Chloro-4'-biphenylol	28034-99-3											0.0365	(2)		

Median/Single RBA Values for Sustances Tested in Two or More In Vitro ER Binding Assays

Substance	CASRN	RUC		hERα		hERα-FP		hERβ		rERβ		GST-aERdef		GST-cERdef	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
11β-Chloromethylestradiol	71794-60-0														
Chrysene	218-01-9	neg (10)	(1)					neg (10)	(1)						
Chrysin	480-40-0	neg (100)	(1)	neg (10)	(1)			neg (10)	(1)						
trans -Clomiphene	911-45-5			25	(1)					12	(1)				
Corticosterone	50-22-6	neg (100)	(1)	neg (100)	(1)					neg (100)	(1)				
Coumestrol	479-13-0	1.86	(2)	34	(3)	12	(1)	120	(2)	185	(1)	3.1	(1)	0.7	(1)
Cyclofenil diphenol	5189-40-2														
Daidzein	486-66-8	0.023	(1)	0.1	(3)	0.2	(1)	1	(3)						
o,p' -DDD	53-19-0	0.009	(1/2)	neg (50)	(2)			neg (10)	(1)						
p,p '-DDD	72-54-8	0.0003	(1/3)	0.009	(1/2)			neg (10)	(1)						
o,p '-DDE	3424-82-6	0.0009	(1/2)	neg (10)	(1)			neg (10)	(1)			neg (100)	(1)	neg (100)	(1)
p,p '-DDE	72-55-9	0.0003	(1/3)	neg (10)	(1)			neg (10)	(1)			neg (100)	(1)	neg (100)	(1)
o,p '-DDT	789-02-6	0.013	(5)	0.055	(2)	0.4	(1)	0.02	(1)			neg (100)	(1)	0.086	(1)
(-)-*o,p* '-DDT	58633-26-4	0.029	(1)												
(+)-*o,p* '-DDT	58633-27-5	neg (20)	(1)												
p,p '-DDT	50-29-3	0.0003	(1/3)	neg (10)	(1)	0.041	(1)	neg (10)	(1)			neg (100)	(1)	neg (100)	(1)
Dehydroepiandrosterone	53-43-0			0.04	(1)					0.07	(1)	neg (100)	(1)	neg (100)	(1)
3-Deoxyestradiol	2529-64-8	0.5	(1)												
3-Deoxyestradiol	2529-64-8														
17-Desoxyestradiol	53-63-4	18.16	(3)												
17-Desoxyestradiol	53-63-4	18.2	(3)												
Dibutyl phthalate	84-74-2	0.0027	(1)			neg (5000)	(1)								
Dibutyl benzyl phthalate												neg (100)	(1)	neg (100)	(1)
2',5'-Dichloro-4-biphenylol	53905-28-5	0.036	(1)	0.033	(1)										
Dieldrin	60-57-1	neg (100)	(2)	neg (10)	(1)	0.0005	(1)								
Dienestrol	84-17-3	37.5	(1)	223	(1)					404	(1)				
Diethylhexyl phthalate	117-81-7	neg (1000)	(2)			neg (5000)	(1)								
Diethylstilbestrol	56-53-1	200	(11)	236	(3)	124	(4)	160.5	(2)	295	(1)	107	(1)	130	(1)
3,3'-Diethylstilbestrol	5959-71-7														
(*rac*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol				14	(1)			67	(1)						
5,11-*trans* -Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol				221	(1)			432	(1)						
(5*R* ,11*R*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol				23	(1)			144	(1)						
(5*S* ,11*S*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol				0.9	(1)			14	(1)						
Dihydrogenistein				0.143	(1)			18.5	(1)						
5α-Dihydrotestosterone	521-18-6	0.0135	(2)	0.05	(1)	0.0095	(1)			0.17	(1)	0.38	(1)	0.0085	(1)
4,4'-Dihydroxybiphenyl	92-88-6			neg (10)	(1)			0.03	(1)						
α,α-Dimethylstilbestrol	552-80-7	14.5	(1)												

Median/Single RBA Values for Sustances Tested in Two or More In Vitro ER Binding Assays

Substance	CASRN	GST-hERαdef		GST-mERαdef		GST-rtERdef		MCF-7 cells		MCF-7 cytosol		MUC		RBC	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
11β-Chloromethylestradiol	71794-60-0							96	(1)	100	(1)				
Chrysene	218-01-9	neg (10)	(1)					13	(1)						
Chrysin	480-40-0														
trans -Clomiphene	911-45-5													0.0081	(1)
Corticosterone	50-22-6														
Coumestrol	479-13-0	0.81	(1)	0.33	(1)	0.24	(1)	12	(1)	13	(1)				
Cyclofenil diphenol	5189-40-2							0.5	(1)	5	(1)				
Daidzein	486-66-8														
o,p' -DDD	53-19-0														
p,p '-DDD	72-54-8														
o,p '-DDE	3424-82-6	neg (100)	(1)	neg (100)	(1)	0.11	(1)								
p,p '-DDE	72-55-9	neg (100)	(1)	neg (100)	(1)	0.042	(1)							neg (100)	(1)
o,p '-DDT	789-02-6	neg (100)	(1)	0.0073	(1)	0.43	(1)			0.0003	(1)	0.21	(1)	0.00059	(1)
(-)-*o,p* '-DDT	58633-26-4							0.013	(1)						
(+)-*o,p* '-DDT	58633-27-5							0.0001	(1)						
p,p '-DDT	50-29-3	neg (100)	(1)	neg (100)	(1)	0.165	(1)								
Dehydroepiandrosterone	53-43-0	neg (100)	(1)	neg (100)	(1)	0.028	(1)								
3-Deoxyestradiol	2529-64-8							0.6	(1)	8	(1)				
3-Deoxyestradiol	2529-64-8							0.6	(1)	8	(1)				
17-Desoxyestradiol	53-63-4							0.5	(1)	40	(1)				
17-Desoxyestradiol	53-63-4							0.5	(1)	40	(1)				
Dibutyl phthalate	84-74-2													neg (100)	(1)
Dibutyl benzyl phthalate		neg (100)	(1)	neg (100)	(1)	0.2	(1)								
2',5'-Dichloro-4-biphenylol	53905-28-5											0.459	(2)		
Dieldrin	60-57-1											neg (10)	(1)		
Dienestrol	84-17-3														
Diethylhexyl phthalate	117-81-7														
Diethylstilbestrol	56-53-1	91	(1)	84	(1)	165	(1)	84	(1)	100	(2)	113	(7)	1551.5	(2)
3,3'-Diethylstilbestrol	5959-71-7							17.5	(1)	3	(1)				
(*rac*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol															
5,11-*trans* -Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol															
(5*R* ,11*R*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol															
(5*S* ,11*S*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol															
Dihydrogenistein															
5α-Dihydrotestosterone	521-18-6	0.049	(1)	0.04	(1)	0.034	(1)					neg (10)	(1)		
4,4'-Dihydroxybiphenyl	92-88-6											neg (5)	(1)		
α,α-Dimethylstilbestrol	552-80-7													129	(1)

Median/Single RBA Values for Sustances Tested in Two or More In Vitro ER Binding Assays

Substance	CASRN	RUC		hERα		hERα-FP		hERβ		rERβ		GST-aERdef		GST-cERdef	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
5,11-*trans* -Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol				222	(1)			254	(1)						
(5*R* ,11*R*)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol				24	(1)			76	(1)						
(5*S* ,11*S*)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol				9.3	(1)			75	(1)						
Phenol, 4-(1, 2-diphenyl-1-butenyl)-	69967-79-9														
Phenol, 4-(1Z) 1-2-diphenyl-1-butenyl)-	69967-80-2														
5,11-*trans* -Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol				33.6	(1)			92.3	(1)						
(5*R,* 11*R*)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol				5.2	(1)			26	(1)						
(5*S* ,11*S*)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol				1.6	(1)			5.1	(1)						
Droloxifene	82413-20-5	15.2	(1)												
α,β-Endosulfan	115-29-7	0.00044	(1/2)	neg (10)	(1)			neg (10)	(1)						
α-Endosulfan	959-98-8			neg (10)	(1)							neg (100)	(1)	neg (100)	(1)
β-Endosulfan	33213-65-9			neg (10)	(1)							neg (100)	(1)	neg (100)	(1)
17-Epiestriol	1228-72-4			29.0	(1)			80	(1)						
Equol	531-95-3	0.15	(1)	0.33	(1)			58.8	(1)						
16α-Estradiol	1090-04-6														
17α-Estradiol	57-91-0	26.5	(2)	32.5	(2)			2	(1)	11	(1)				
Estradiol 3-benzoate	50-50-0											13	(1)	15	(1)
17β-Estradiol 3-methyl ether	1035-77-4	0.7	(1)												
9-Estratetraene-3,17β-diol	791-69-5														
Estra-1,3,5(10)-triene-3,17β-diol, 14,15α-epoxy-	79581-12-7														
Estra-1,3,5(10)-triene-3,17β-diol, 14β,15β-epoxy-	79645-49-1														
Estra-1,3,5,(10),trien-3,14,17β-triol	16288-09-8														
Estriol	50-27-1	14.4	(2)	14	(1)					21	(1)	30	(1)	11	(1)
Estrone	53-16-7	48	(4)	60	(1)	2.1	(1)			37	(1)	60	(1)	50	(1)
Estrone 3-sulfate	481-97-0			neg (100)	(1)					neg (100)	(1)				
17α-Ethinyl estradiol	57-63-6	173	(4)									139	(1)	171	(1)
Flavone	525-82-6	neg (100)	(1)	neg (10)	(1)			neg (10)	(1)						
Formononetin	485-72-3	0.0013	(1)	neg (10)	(1)			neg (10)	(1)						
Genistein	446-72-0	0.56	(2)	2.36	(4)	1.6	(1)	13	(3)	36	(1)	1.3	(1)	0.78	(1)
Genistin	529-59-9	neg (100)	(1)					0.014	(1)						
Glycitein	40957-83-3			0.016	(1)			0.91	(1)						
2,2',3,3',4',5,6-Heptachlorobiphenyl	52663-70-4											neg (10)	(1)		
2,2',3,3',4,5,6,-Heptachlorobiphenyl	68194-16-1											neg (10)	(1)		
2,2',3,3',5,5',6-Heptachlorobiphenyl	52663-64-6											neg (10)	(1)		
2,2',3,4,4',5',6-Heptachlorobiphenyl	52663-69-1											neg (10)	(1)		
2,2',3,4,4',6,6'-Heptachlorobiphenyl	74472-48-3											0.025	(1)		

Median/Single RBA Values for Sustances Tested in Two or More In Vitro ER Binding Assays

Substance	CASRN	GST-hERαdef		GST-mERαdef		GST-rtERdef		MCF-7 cells		MCF-7 cytosol		MUC		RBC	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
5,11-*trans* -Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol															
(5*R* ,11*R*)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol															
(5*S* ,11*S*)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol															
Phenol, 4-(1, 2-diphenyl-1-butenyl)-	69967-79-9							2	(1)	15	(1)				
Phenol, 4-(1Z) 1-2-diphenyl-1-butenyl)-	69967-80-2							0.4	(1)	1	(1)				
5,11-*trans* -Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol															
(5*R,* 11*R*)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol															
(5*S* ,11*S*)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol															
Droloxifene	82413-20-5							0.2	(1)	2.5	(1)				
α,β-Endosulfan	115-29-7											neg (5)	(1)	neg (100)	(1)
α-Endosulfan	959-98-8	neg (100)	(1)	neg (100)	(1)	0.012	(1)								
β-Endosulfan	33213-65-9	neg (100)	(1)	neg (100)	(1)	neg (100)	(1)			0.00024	(1)				
17-Epiestriol	1228-72-4														
Equol	531-95-3														
16α-Estradiol	1090-04-6									17.9	(2)			66	(1)
17α-Estradiol	57-91-0									0.22	(1)	1000	(1)	49	(1)
Estradiol 3-benzoate	50-50-0	10	(1)	12	(1)	9	(1)								
17β-Estradiol 3-methyl ether	1035-77-4													3	(1)
9-Estratetraene-3,17β-diol	791-69-5							37	(1)	80	(1)				
Estra-1,3,5(10)-triene-3,17β-diol, 14,15α-epoxy-	79581-12-7							10	(1)	5	(1)				
Estra-1,3,5(10)-triene-3,17β-diol, 14β,15β-epoxy-	79645-49-1							0.08	(1)	neg (0.1)	(1)				
Estra-1,3,5,(10),trien-3,14,17β-triol	16288-09-8							1.5	(1)	2	(1)				
Estriol	50-27-1	28	(1)	13	(1)	3.7	(1)	20	(1)	13	(3)	100	(1)	16	(1)
Estrone	53-16-7	45	(1)	28	(1)	14	(1)	19	(1)	13	(3)	100	(1)	66	(1)
Estrone 3-sulfate	481-97-0														
17α-Ethinyl estradiol	57-63-6	127	(1)	118	(1)	108	(1)							191	(1)
Flavone	525-82-6														
Formononetin	485-72-3														
Genistein	446-72-0	0.46	(1)	0.33	(1)	0.44	(1)			2.31	(2)				
Genistin	529-59-9														
Glycitein	40957-83-3														
2,2',3,3',4',5,6-Heptachlorobiphenyl	52663-70-4	neg (10)	(1)			0.031	(1)								
2,2',3,3',4,5,6,-Heptachlorobiphenyl	68194-16-1	neg (10)	(1)			0.031	(1)								
2,2',3,3',5,5',6-Heptachlorobiphenyl	52663-64-6	neg (10)	(1)			neg (10)	(1)								
2,2',3,4,4',5',6-Heptachlorobiphenyl	52663-69-1	neg (10)	(1)			neg (10)	(1)								
2,2',3,4,4',6,6'-Heptachlorobiphenyl	74472-48-3	0.024	(1)			0.78	(1)								

Median/Single RBA Values for Sustances Tested in Two or More In Vitro ER Binding Assays

Substance	CASRN	RUC		hERα		hERα-FP		hERβ		rERβ		GST-aERdef		GST-cERdef	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0											neg (10)	(1)		
2,2',3,4',5,6,6'-Heptachlorobiphenyl	74487-85-7											0.025	(1)		
2,3,3',4,4',5,6-Heptachlorobiphenyl	41411-64-7											neg (10)	(1)		
2,3,3',4',5,5',6-Heptachlorobiphenyl	69782-91-8											neg (10)	(1)		
2,2',3,3',4',5,5'-Heptachloro-4-biphenylol	158076-64-3			0.1	(1)			0.1	(1)			neg (10)	(1)		
2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8			0.09	(2)			0.1	(2)			neg (10)	(1)		
2,2',3,4',5,5',6-Heptachloro-4-biphenylol	158076-68-7			0.1	(1)			0.1	(1)			neg (10)	(1)		
2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3											neg (10)	(1)		
2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2											neg (10)	(1)		
2,2',3,4',5',6-Hexachlorobiphenyl	38380-04-0											neg (10)	(1)		
2,2',3,4,5,6'-Hexachlorobiphenyl	68194-15-0											neg (10)	(1)		
2,2',3,5,5',6-Hexachlorobiphenyl	52663-63-5											neg (10)	(1)		
2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1											neg (10)	(1)		
2,3,3',4,4',6-Hexachlorobiphenyl	74472-42-7											neg (10)	(1)		
2,3',4,4',5',6-Hexachlorobiphenyl	59291-65-5											neg (10)	(1)		
2,2',4,4',6,6'-Hexachlorobiphenyl	33979-03-2			neg (50)	(1)										
3,3',4,4',5,5'-Hexachlorobiphenyl	32774-16-6											neg (10)	(1)		
2,2',3,3',4',5-Hexachloro-4-biphenylol	158076-62-1			0.07	(1)			0.06	(1)			neg (10)	(1)		
2,2',3,4',5,5'-Hexachloro-4-biphenylol	145413-90-7			0.03	(1)			0.04	(1)			neg (10)	(1)		
Hexestrol	84-16-2	299.8	(2)	302	(1)					234	(1)				
3-Hydroxybenzo[*b*]naphtho[2,1-d]thiophene								2.5	(1)						
2-Hydroxybenzo[*c*]phenanthrene								3.1	(1)						
3-Hydroxybenzo[*b*]phenanthro[2,3-d]thiophene								5	(1)						
2-Hydroxychrysene	65945-06-4							13.33	(1)						
11β-Hydroxyestradiol	5444-22-4	7	(1)												
14β-Hydroxyestradiol	60183-66-6														
2-Hydroxyestradiol	362-05-0			7	(1)					11	(1)				
4-Hydroxyestradiol	5976-61-4			13	(1)					7	(1)				
2-Hydroxyestrone	362-06-1			2	(1)			0.2	(1)						
2-Hydroxy-5-methylchrysene								19.3	(1)						
8-Hydroxy-5-methylchrysene								3.1	(1)						
4-Hydroxytamoxifen	68047-06-3	175.2	(1)	178	(3)	21.5	(3)	147	(2)	339	(1)	243	(1)	168	(1)
ICI 164384	98007-99-9	14.5	(1)	121.5	(2)			93	(1)	166	(1)	28	(1)	62	(1)
ICI 182780	129453-61-8	235.2	(2)	32	(1)			25	(1)						
(*E*)-17α-Iodovinylestradiol	82123-96-4	7	(1)												
(*Z*)-17α-Iodovinylestradiol	177159-09-0	63	(1)												
Ipriflavone	35212-22-7			neg (10)	(1)			neg (10)	(1)						
Kaempferol	520-18-3	0.025	(1)	0.1	(1)			2.5	(2)						

Median/Single RBA Values for Sustances Tested in Two or More In Vitro ER Binding Assays

Substance	CASRN	GST-hERαdef		GST-mERαdef		GST-rtERdef		MCF-7 cells		MCF-7 cytosol		MUC		RBC	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0	neg (10)	(1)			neg (10)	(1)								
2,2',3,4',5,6,6'-Heptachlorobiphenyl	74487-85-7	0.024	(1)			0.24	(1)								
2,3,3',4,4',5,6-Heptachlorobiphenyl	41411-64-7	neg (10)	(1)			neg (10)	(1)								
2,3,3',4',5,5',6-Heptachlorobiphenyl	69782-91-8	neg (10)	(1)			neg (10)	(1)								
2,2',3,3',4',5,5'-Heptachloro-4-biphenylol	158076-64-3	neg (10)	(1)			neg (10)	(1)								
2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8	neg (10)	(1)			neg (10)	(1)								
2,2',3,4',5,5',6-Heptachloro-4-biphenylol	158076-68-7	neg (10)	(1)			neg (10)	(1)								
2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3	neg (10)	(1)			neg (10)	(1)								
2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2	neg (10)	(1)			neg (10)	(1)								
2,2',3,4',5',6-Hexachlorobiphenyl	38380-04-0	neg (10)	(1)			neg (10)	(1)								
2,2',3,4,5,6'-Hexachlorobiphenyl	68194-15-0	neg (10)	(1)			0.031	(1)								
2,2',3,5,5',6-Hexachlorobiphenyl	52663-63-5	neg (10)	(1)			neg (10)	(1)								
2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	neg (10)	(1)			neg (10)	(1)								
2,3,3',4,4',6-Hexachlorobiphenyl	74472-42-7	neg (10)	(1)			neg (10)	(1)								
2,3',4,4',5',6-Hexachlorobiphenyl	59291-65-5	neg (10)	(1)			neg (10)	(1)								
2,2',4,4',6,6'-Hexachlorobiphenyl	33979-03-2											0.27	(1)		
3,3',4,4',5,5'-Hexachlorobiphenyl	32774-16-6	neg (10)	(1)			neg (10)	(1)								
2,2',3,3',4',5-Hexachloro-4-biphenylol	158076-62-1	neg (10)	(1)			neg (10)	(1)								
2,2',3,4',5,5'-Hexachloro-4-biphenylol	145413-90-7	neg (10)	(1)			neg (10)	(1)								
Hexestrol	84-16-2							58	(1)	100	(1)			74	(1)
3-Hydroxybenzo[*b*]naphtho[2,1-d]thiophene		1.83	(1)												
2-Hydroxybenzo[*c*]phenanthrene		2.2	(1)												
3-Hydroxybenzo[*b*]phenanthro[2,3-d]thiophene		2.4	(1)												
2-Hydroxychrysene	65945-06-4	5.8	(1)												
11β-Hydroxyestradiol	5444-22-4							4	(1)	8.9	(2)			4	(1)
14β-Hydroxyestradiol	60183-66-6							3.5	(1)	10	(1)				
2-Hydroxyestradiol	362-05-0														
4-Hydroxyestradiol	5976-61-4														
2-Hydroxyestrone	362-06-1														
2-Hydroxy-5-methylchrysene		19.6	(1)												
8-Hydroxy-5-methylchrysene		3.1	(1)												
4-Hydroxytamoxifen	68047-06-3	155	(1)	212	(1)	272	(1)	2.9	(1)	100	(1)	14.4	(1)		
ICI 164384	98007-99-9	42	(1)	45	(1)	327	(1)								
ICI 182780	129453-61-8													500	(1)
(*E*)-17α-Iodovinylestradiol	82123-96-4									17	(1)				
(*Z*)-17α-Iodovinylestradiol	177159-09-0									51	(1)				
Ipriflavone	35212-22-7														
Kaempferol	520-18-3														

Median/Single RBA Values for Sustances Tested in Two or More In Vitro ER Binding Assays

Substance	CASRN	RUC		hERα		hERα-FP		hERβ		rERβ		GST-aERdef		GST-cERdef	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
Kepone	143-50-0	0.03	(3)	0.06	(1)	0.2	(1)	0.1	(1)			0.011	(1)	0.011	(1)
16-Ketoestradiol	566-75-6			1.3	(1)			0.9	(1)						
6-Ketoestradiol	571-92-6														
Mestranol	72-33-3	1.26	(2)			0.0096	(1)								
Methoxychlor *(p,p'-)*	72-43-5	0.001	(3/5)	0.01	(1/2)			neg (10)	(1)	0.13	(1)	neg (100)	(1)	neg (100)	(1)
(*E*)-11β-methoxy-17α-iodovinylestradiol	90857-55-9	11	(1)												
(*Z*)-11β-Methoxy-17α-iodovinylestradiol	177159-11-4	41	(1)												
11β-Methylestradiol-17β	23637-93-6	124	(1)												
Moxestrol	34816-55-2	13.8	(1)	43	(1)					5	(1)				
Nafoxidine	1845-11-0	0.72	(1)	44	(1)					16	(1)				
Naringenin	480-41-1	0.0075	(1)	0.01	(1)			0.155	(2)			0.065	(1)	0.0082	(1)
4-*n* -Nonylphenol	25154-52-3			0.0255	(2)			0.0765	(2)						
p -Nonylphenol	104-40-5	0.033	(10)			0.4	(2)								
Norethindrone	68-22-4			0.07	(1)					0.01	(1)				
Norethynodrel	68-23-5	0.22	(2)	0.7	(1)					0.22	(1)				
19-Nortestosterone	434-22-0			0.01	(1)					0.23	(1)				
2,2'3,3',4,4',5,5'-Octachlorobiphenyl	35694-08-7											neg (10)	(1)		
4-Octylphenol	1806-26-4	0.005	(1)	0.02	(1)			0.07	(1)						
4-*tert* -Octylphenol	140-66-9	0.197	(3)	0.01	(1)	0.2	(1)	0.03	(1)			0.099	(1)	0.57	(1)
2,2',3,3',6-Pentachlorobiphenyl	52663-60-2											neg (10)	(1)		
2,2',3,4,5'-Pentachlorobiphenyl	38380-02-8											neg (10)	(1)		
2,2',3,4',6-Pentachlorobiphenyl	68194-05-8											neg (10)	(1)		
2,2',3,5',6-Pentachlorobiphenyl	38379-99-6											neg (10)	(1)		
2,2',4,4',5-Pentachlorobiphenyl	38380-01-7											neg (10)	(1)		
2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2											neg (10)	(1)		
2,2',4,6,6'-Pentachlorobiphenyl	56558-16-8											0.025	(1)		
2,3,3',5,6,-Pentachlorobiphenyl	74472-36-9											neg (10)	(1)		
2,3,4,4',6,-Pentachlorobiphenyl	74472-38-1											neg (10)	(1)		
3,3',4,4',5,-Pentachlorobiphenyl	57465-28-8											neg (10)	(1)		
2',3,3',4',5'-Pentachloro-4-biphenylol	149589-55-9	0.082	(1)	0.11	(1)			0.11	(1)						
2,3,3',4',5-Pentachloro-4-biphenylol	152969-11-4			0.03	(1)			0.11	(1)			neg (10)	(1)		
2',3,3',4',6'-Pentachloro-4-biphenylol	192190-10-6	0.041	(1)	0.13	(1)			0.12	(1)						
2,2',3',4',5'-Pentachloro-4-biphenylol	150304-12-4	0.036	(1)	0.1	(1)			0.13	(1)						
2,2',3',4',6'-Pentachloro-4-biphenylol	150304-10-2	0.12	(1)	0.3	(1)			0.2	(1)						
2,2',3',5',6'-Pentachloro-4-biphenylol	150304-11-3	0.14	(1)	0.09	(1)			0.03	(1)						
2',3,3',4',5-Pentachloro-4-biphenylol	192190-09-3			0.01	(1)			neg (10)	(1)						
2',3,3',5',6'-Pentachloro-4-biphenylol	189578-02-7	0.068	(1)	0.06	(1)			0.04	(1)						
Phenol, 4,4'-[1,2-bis(methylene)-1,2-ethanediyl]bis	107144-81-0														

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Substance	CASRN	GST-hERαdef		GST-mERαdef		GST-rtERdef		MCF-7 cells		MCF-7 cytosol		MUC		RBC	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
Kepone	143-50-0	0.0069	(1)	0.0035	(1)	0.054	(1)					neg (5)	(1)		
16-Ketoestradiol	566-75-6									0.9	(1)			14	(1)
6-Ketoestradiol	571-92-6							15	(1)	20	(1)				
Mestranol	72-33-3													8	(1)
Methoxychlor *(p,p'-)*	72-43-5	neg (100)	(1)	neg (100)	(1)	0.95	(1)			neg (1000)	(1)	neg (5)	(1)	0.0031	(1)
(*E*)-11β-methoxy-17α-iodovinylestradiol	90857-55-9									17	(1)				
(*Z*)-11β-Methoxy-17α-iodovinylestradiol	177159-11-4									31	(1)				
11β-Methylestradiol-17β	23637-93-6							100	(1)	100	(1)				
Moxestrol	34816-55-2											4.25	(1)		
Nafoxidine	1845-11-0							0.1	(1)	5	(1)				
Naringenin	480-41-1	neg (100)	(1)	neg (100)	(1)	0.039	(1)								
4-*n* -Nonylphenol	25154-52-3											neg (5)	(1)	0.0011	(1)
p -Nonylphenol	104-40-5							0.026	(1)	0.021	(1)				
Norethindrone	68-22-4														
Norethynodrel	68-23-5														
19-Nortestosterone	434-22-0											0.1	(1)		
2,2'3,3',4,4',5,5'-Octachlorobiphenyl	35694-08-7	neg (10)	(1)			neg (10)	(1)								
4-Octylphenol	1806-26-4							0.072	(1)	1	(1)			neg (100)	(1)
4-*tert* -Octylphenol	140-66-9	0.12	(1)	0.17	(1)	3.2	(1)								
2,2',3,3',6-Pentachlorobiphenyl	52663-60-2	neg (10)	(1)			neg (10)	(1)								
2,2',3,4,5'-Pentachlorobiphenyl	38380-02-8	neg (10)	(1)			neg (10)	(1)								
2,2',3,4',6-Pentachlorobiphenyl	68194-05-8	neg (10)	(1)			0.031	(1)								
2,2',3,5',6-Pentachlorobiphenyl	38379-99-6	neg (10)	(1)			neg (10)	(1)								
2,2',4,4',5-Pentachlorobiphenyl	38380-01-7	neg (10)	(1)			neg (10)	(1)								
2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2	neg (10)	(1)			neg (10)	(1)								
2,2',4,6,6'-Pentachlorobiphenyl	56558-16-8	0.024	(1)			0.24	(1)					0.88	(1)		
2,3,3',5,6,-Pentachlorobiphenyl	74472-36-9	neg (10)	(1)			neg (10)	(1)								
2,3,4,4',6,-Pentachlorobiphenyl	74472-38-1	neg (10)	(1)			0.031	(1)								
3,3',4,4',5,-Pentachlorobiphenyl	57465-28-8	neg (10)	(1)			neg (10)	(1)								
2',3,3',4',5'-Pentachloro-4-biphenylol	149589-55-9											0.072	(1)		
2,3,3',4',5-Pentachloro-4-biphenylol	152969-11-4	neg (10)	(1)			neg (10)	(1)			1	(1)				
2',3,3',4',6'-Pentachloro-4-biphenylol	192190-10-6											neg (1000)	(1)		
2,2',3',4',5'-Pentachloro-4-biphenylol	150304-12-4											0.033	(1)		
2,2',3',4',6'-Pentachloro-4-biphenylol	150304-10-2											0.044	(1)		
2,2',3',5',6'-Pentachloro-4-biphenylol	150304-11-3											0.013	(1)		
2',3,3',4',5-Pentachloro-4-biphenylol	192190-09-3					neg (10)	(1)			0.1	(2)	0.072	(1)		
2',3,3',5',6'-Pentachloro-4-biphenylol	189578-02-7											0.031	(1)		
Phenol, 4,4'-[1,2-bis(methylene)-1,2-ethanediyl]bis	107144-81-0							26	(1)	20	(1)				

Median/Single RBA Values for Sustances Tested in Two or More In Vitro ER Binding Assays

Substance	CASRN	RUC		hERα		hERα-FP		hERβ		rERβ		GST-aERdef		GST-cERdef	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
Phenol, 4-[7-[2 dimethylamino)ethoxy]-11-ethyldibenzo[*b,f*]thiepin-10-yl)	85850-74-4	63	(1)												
Phenol, 4-[3-[2 dimethylamino)ethoxy]-11-ethyldibenzo[*b,f*]thioctin-12-yl)	85850-81-3	52	(1)												
Phenol, 4-[2-[2 dimethylamino)ethoxy]-6-ethyl-11,12-dihydrodibenzo[*a,e*]cycloocten-5-yl]	85870-75-5	50	(1)												
Phenol, 3-[2-dimethylamino-ethoxy]-10-ethyl-4-hydroxy-phenyl dibenzo-[b,f]oxepin	85850-80-2	6.1	(1)												
Phenol, 4-[1-[4-[2-(dimethylamino)ethoxy] phenyl]-2-phenyl-1-butenyl]-3-methyl-, (*E*)	96474-35-0														
Phenol, 4-[2-nitro-2-phenyl-1-[4-[2-(1-pyrrolidinyl)ethoxy] phenyl]-ethenyl]	107144-84-3														
Phenol, 4,4'-(2-phenyl-1-butenylidene)bis-	91221-46-4														
4-Phenylphenol	92-69-3	0.001	(1)												
Phloretin	60-82-2	0.069	(1)	0.2	(1)			0.7	(1)						
Progesterone	57-83-0	0.0003	(1/3)	neg (100)	(2)			neg (10)	(1)	neg (100)	(1)				
Promegestone	34184-77-5	0.22	(1/2)												
Propylpyrazoletriol				49	(1)			0.12	(1)						
Pyrrolidine, 1-[2-[4-[1-(4-methoxyphenyl)-2-nitro-2-phenylethenyl]phenoxy]ethyl]-, (*E*) (9 CI)	77413-87-7														
Quercetin	117-39-5	neg (100)	(1)	0.0100	(1)			0.04	(1)			0.016	(1)	0.0039	(1)
6-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl) 4-methyl-	107144-82-1														
7-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl) 4-methyl-	107144-83-2														
Raloxifene	84449-90-1			60.4	(2)			21	(2)						
Simazine	122-34-9	neg (33.3)	(1)			neg (2000)	(1)					neg (100)	(1)	neg (100)	(1)
ß-Sitosterol	83-46-5	neg (1000)	(1)	neg (100)	(1)					neg (100)	(1)	neg (100)	(1)	neg (100)	(1)
Tamoxifen	10540-29-1	3.1	(4)	4	(3)	3.03	(2)	2.34	(2)	6	(1)	10	(1)	16	(1)
Testosterone	58-22-0	0.01	(1/2)	neg (100)	(2)			neg (10)	(1)	neg (100)	(1)				
2,2',3,3'-Tetrachlorobiphenyl	3844-93-8											neg (10)	(1)		
2,2',3,4-Tetrachlorobiphenyl	52663-59-9											neg (10)	(1)		
2,2',3,6-Tetrachlorobiphenyl	41464-47-5											neg (10)	(1)		
2,2',4,4'-Tetrachlorobiphenyl	2437-79-8	neg (100)	(1)									neg (10)	(1)		
2,2',4,5'-Tetrachlorobiphenyl	41464-40-8											neg (10)	(1)		
2,2',4,6'-Tetrachlorobiphenyl	68194-04-7											neg (10)	(1)		
2,2',6,6'-Tetrachlorobiphenyl	15968-05-5			neg (100)	(1)			neg (100)	(1)			neg (10)	(1)		
2,3,3',5'-Tetrachlorobiphenyl	41464-49-7											neg (10)	(1)		
2,3,4,4'-Tetrachlorobiphenyl	33025-41-1											neg (10)	(1)		
2,3',4',5-Tetrachlorobiphenyl	32598-11-1											neg (10)	(1)		
2,3',4,5'-Tetrachlorobiphenyl	73575-52-7											neg (10)	(1)		
2,4,4',5-Tetrachlorobiphenyl	32690-93-0											neg (10)	(1)		
3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	neg (300)	(1)	neg (50)	(1)							neg (10)	(1)		

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Substance	CASRN	GST-hERαdef		GST-mERαdef		GST-rtERdef		MCF-7 cells		MCF-7 cytosol		MUC		RBC	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
Phenol, 4-[7-[2 dimethylamino)ethoxy]-11-ethyldibenzo[*b,f*]thiepin-10-yl)	85850-74-4							0.3	(1)	63	(1)				
Phenol, 4-[3-[2 dimethylamino)ethoxy]-11-ethyldibenzo[*b,f*]thioctin-12-yl)	85850-81-3							2.5	(1)	50	(1)				
Phenol, 4-[2-[2 dimethylamino)ethoxy]-6-ethyl-11,12-dihydrodibenzo[*a,e*]cycloocten-5-yl]	85870-75-5							1.3	(1)	50	(1)				
Phenol, 3-[2-dimethylamino-ethoxy]-10-ethyl-4-hydroxy-phenyl dibenzo-[b,f]oxepin	85850-80-2							0.1	(1)	6	(1)				
Phenol, 4-[1-[4-[2-(dimethylamino)ethoxy] phenyl]-2-phenyl-1-butenyl]-3-methyl-, (*E*)	96474-35-0							0.4	(1)	100	(1)				
Phenol, 4-[2-nitro-2-phenyl-1-[4-[2-(1-pyrrolidinyl)ethoxy] phenyl]-ethenyl]	107144-84-3							2.1	(1)	100	(1)				
Phenol, 4,4'-(2-phenyl-1-butenylidene)bis-	91221-46-4							2	(1)	100	(1)				
4-Phenylphenol	92-69-3											neg (5)	(1)		
Phloretin	60-82-2														
Progesterone	57-83-0											neg (10)	(1)		
Promegestone	34184-77-5											neg (10)	(1)		
Propylpyrazoletriol															
Pyrrolidine, 1-[2-[4-[1-(4-methoxyphenyl)-2-nitro-2-phenylethenyl]phenoxy]ethyl]-, (*E*) (9 CI)	77413-87-7							0.07	(1)	11	(1)				
Quercetin	117-39-5	neg (100)	(1)	neg (100)	(1)	0.042	(1)			neg (25)	(1)				
6-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-	107144-82-1							22	(1)	33	(1)				
7-Quinolinol, 1-ethyl-1,2-dihydro-3-(4-hydroxyphenyl)-4-methyl-	107144-83-2							3	(1)	9	(1)				
Raloxifene	84449-90-1														
Simazine	122-34-9	neg (100)	(1)	neg (100)	(1)	neg (100)	(1)								
ß-Sitosterol	83-46-5	neg (100)	(1)	neg (100)	(1)	neg (100)	(1)								
Tamoxifen	10540-29-1	11	(1)	10	(1)	25	(1)	0.06	(1)	1	(1)	6.55	(1)	0.017	(1)
Testosterone	58-22-0											neg (10)	(1)	neg (100)	(1)
2,2',3,3'-Tetrachlorobiphenyl	3844-93-8	neg (10)	(1)			neg (10)	(1)								
2,2',3,4-Tetrachlorobiphenyl	52663-59-9	neg (10)	(1)			0.024	(1)								
2,2',3,6-Tetrachlorobiphenyl	41464-47-5	neg (10)	(1)			0.031	(1)								
2,2',4,4'-Tetrachlorobiphenyl	2437-79-8	neg (10)	(1)			0.031	(1)								
2,2',4,5'-Tetrachlorobiphenyl	41464-40-8	neg (10)	(1)			neg (10)	(1)								
2,2',4,6'-Tetrachlorobiphenyl	68194-04-7	neg (10)	(1)			0.0310	(1)								
2,2',6,6'-Tetrachlorobiphenyl	15968-05-5	neg (10)	(1)			0.0310	(1)								
2,3,3',5'-Tetrachlorobiphenyl	41464-49-7	neg (10)	(1)			neg (10)	(1)								
2,3,4,4'-Tetrachlorobiphenyl	33025-41-1	neg (10)	(1)			neg (10)	(1)								
2,3',4',5-Tetrachlorobiphenyl	32598-11-1	neg (10)	(1)			neg (10)	(1)								
2,3',4,5'-Tetrachlorobiphenyl	73575-52-7	neg (10)	(1)			neg (10)	(1)								
2,4,4',5-Tetrachlorobiphenyl	32690-93-0	neg (10)	(1)			neg (10)	(1)								
3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	neg (10)	(1)			neg (10)	(1)								

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Substance	CASRN	RUC		hERα		hERα-FP		hERβ		rERβ		GST-aERdef		GST-cERdef	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
3,3',4,5-Tetrachlorobiphenyl	70362-49-1											neg (10)	(1)		
3,3',5,5'-Tetrachloro-4,4'-biphenyldiol	13049-13-3	0.001	(1)												
2,2',4',6'-Tetrachloro-4-biphenylol	150304-08-8	0.0005	(1)	0.3	(1)			0.5	(1)						
2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8			0.3	(3)			0.25	(1)			0.5	(1)		
2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	0.23	(1)	3.4	(1)			7.2	(1)			0.5	(1)		
2',3,4',6'-Tetrachloro-4-biphenylol	189578-00-5	neg (1000)	(1)	0.18	(1)			0.23	(1)						
Tetrahydrochrysene	104460-72-2			3	(1)			6.5	(1)						
(rac) -Tetrahydrochrysene				2.5	(1)			25	(1)						
(*R,R*)-Tetrahydrochrysene				3.6	(1)			25	(1)						
(S,S) -Tetrahydrochrysene				0.83	(1)			1.3	(1)						
Tetramethylhexestrol	74385-27-6														
Toxaphene	8001-35-2			neg (10)	(1)										
triaryl-Pyrazole				60	(1)			18	(1)						
2,2',5-Trichlorobiphenyl	37680-65-2											neg (10)	(1)		
2,4,6-Trichloro-4'-biphenylol	35693-92-6			0.747	(1)										
2',4',6'-Trichloro-4-biphenylol	14962-28-8			1.58	(2)			4.7	(1)						
β-Zearalanol	42422-68-4	0.64	(1)	16	(1)					14	(1)				
α-Zearalenol	36455-72-8	43	(1)									36	(1)	70	(1)
β-Zearalenol	71030-11-0	0.2	(1)									4.2	(1)	23	(1)
Zearalenone	17924-92-4	44.1	(1)	8.5	(2)	22	(1)	11.5	(2)			12	(1)	33	(1)

RBA data presented as median RBA value (in micromolar) for two or more experiments using the same assay, or as a a single value if only one experiment had been conducted. Neg = negative for ER binding; number in parenthesis indicates the highest dose tested.

n = the number of experiments conducted. If two numbers are in parenthesis, the first number indicates the number of positive experiments, while the second number indicates the number of experiments conducted.

Median/Single RBA Values for Sustances Tested in Two or More In Vitro ER Binding Assays

Substance	CASRN	GST-hERαdef		GST-mERαdef		GST-rtERdef		MCF-7 cells		MCF-7 cytosol		MUC		RBC	
		RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n	RBA	n
3,3',4,5-Tetrachlorobiphenyl	70362-49-1	neg (10)	(1)			neg (10)	(1)								
3,3',5,5'-Tetrachloro-4,4'-biphenyldiol	13049-13-3									0.4	(1)	0.102	(2)		
2,2',4',6'-Tetrachloro-4-biphenylol	150304-08-8											0.018	(1)		
2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8	0.48	(1)			1.03	(1)								
2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	2.4	(1)			1.15	(1)			3.2	(1)	1.11	(3)		
2',3,4',6'-Tetrachloro-4-biphenylol	189578-00-5											neg (1000)	(1)		
Tetrahydrochrysene	104460-72-2														
(rac) -Tetrahydrochrysene															
(*R,R*)-Tetrahydrochrysene															
(S,S) -Tetrahydrochrysene															
Tetramethylhexestrol	74385-27-6							2	(1)	1.5	(1)				
Toxaphene	8001-35-2									0.0003	(1)	neg (10)	(1)		
triaryl-Pyrazole															
2,2',5-Trichlorobiphenyl	37680-65-2	neg (10)	(1)			neg (10)	(1)								
2,4,6-Trichloro-4'-biphenylol	35693-92-6											5.37	(1)		
2',4',6'-Trichloro-4-biphenylol	14962-28-8									6.3	(1)	2.38	(3)		
β-Zearalanol	42422-68-4														
α-Zearalenol	36455-72-8	48	(1)	53	(1)	267	(1)								
β-Zearalenol	71030-11-0	13	(1)	11	(1)	91	(1)								
Zearalenone	17924-92-4	9.3	(1)	12	(1)	82	(1)			18	(1)				

RBA data presented as median RBA value (in micromolar) for two or more experiments using the same assay, or as a a single value if only one experiment had been conducted. Neg = negative for ER binding; number in parenthesis indicates the highest dose tested.
n = the number of experiments conducted. If two numbers are in parenthesis, the first number indicates the number of positive experiments, while the second number indicates the number of experiments conducted.