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.

HTML BRD Page

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

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LIST OF ACRONYMS AND ABBREVIATIONS

AF	Activation function
ANOVA	Analysis of variance
AR	Androgen receptor
ATP	Adenosine triphosphate
β-*gal*	gene for β-galactosidase
BRD	Background Review Document
CASRN	Chemical Abstracts Service Registry Number
CAT	Chloramphenicol acetyl transferase
cDNA	Complimentary deoxyribonucleic acid
CDTA	*trans*-1,2-diaminocyclohexane–N,N,N',N'-tetraacetic acid
CHO	Chinese hamster ovary
CMA	Chemical Manufacturers Association
CoA	Co-enzyme A
CP	Cell proliferation
CRGP	Chlorophenol red-b-D-galactopyranoside
CUP1	Copper-responsive yeast metallothionein
CYC1	Iso-1-cytochrome C promoter
DDT	Dichlorodiphenyltrichloroethane
DEAE	2-(Diethylamino)ethyl
def	Ligand binding domain of the estrogen receptor
DMSO	Dimethyl sulfoxide
EC_{50}	Half-maximal effective concentration
EDSP	Endocrine Disruptor Screening Program
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee
EPA	Environmental Protection Agency
ER	Estrogen receptor
ERα	Estrogen receptor alpha
ERβ	Estrogen receptor beta
ERE	Estrogen response element

FBS	Fetal bovine serum
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FFDCA	Federal Food, Drug, & Cosmetic Act
FQPA	Food Quality Protection Act
FR	Federal Register
GLP	Good Laboratory Practices
GR	Glucocorticoid receptor
hER	Human estrogen receptor
HPTE	2,2-Bis-(*p*-chlorophenyl)-1,1,1-trichloroethane
HRE	Hormone responsive elements
IC_{50}	Concentration of the test substance inhibiting the reference estrogen response by 50%
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
luc	Luciferase reporter gene
µL	Microliter
µM	Micromolar
mER	Mouse estrogen receptor
mM	Millimolar
MMTV	Mouse mammary tumor virus
mRNA	Messenger RNA
NAS	National Academy of Sciences
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
ng	Nanogram
nm	Nanometer
nM	Nanomolar
OD	Optical density
OECD	Organisation for Economic Co-operation and Development
OPNG	*o*-Nitrophenylgalactoside
PCB	Polychlorinated biphenyls

pgk	Phosphoglycerate kinase
pM	Picomolar
QC	Quality control
QSAR	Quantitative structure activity relationship
r	Rat
RPE	Relative proliferative effect
RPP	Relative proliferative potency
rt	Rainbow trout
SAB	Science Advisory Board
SAP	Scientific Advisory Panel
SDS	Sodium dodecyl sulfate
SDWA	Safe Drinking Water Act
SV-40	Simian virus 40
TA	Transcriptional activation
TIF	Transcriptional intermediary factors
tk	thymidine kinase
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 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), and 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 transcriptional activation (TA) 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* ER binding assays, *in vitro* AR binding assays, and *in vitro* AR TA 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 TA assay. It was envisioned that these criteria would be based on the performance of existing standardized *in vitro* ER TA assays. The minimum performance criteria could then be used to assess the acceptability of new or revised assays proposed in the future. However, a comprehensive review determined that there were no standardized *in vitro* ER TA assays with adequate validation data that could

serve as the basis for establishing these performance criteria. An independent Expert Panel (Panel) will therefore be convened to assess the status of existing *in vitro* ER TA 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 TA 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 TA assays.

This BRD reviews available *in vitro* ER TA assays and presents the data available for substances evaluated in these assays. The relative performance of various types of *in vitro* ER TA 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 TA 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 TA 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, Apr. 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 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 will be 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 (October 2002). A final ICCVAM Test Method Evaluation report will be published in early 2003. This report will include 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 initiate transcriptional activation of the estrogen receptor (*in vitro* ER TA assays); (2) assess the *in vitro* ER TA assays considered for their effectiveness in identifying endocrine-active substances; (3) identify and prioritize *in vitro* ER TA 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 TA 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 identified 258 records related to *in vitro* ER TA assays with 86 publications containing relevant data on ER-induced transcriptional activation for inclusion in this BRD. Data from two submitted non-peer reviewed documents were included also. Some of the peer-reviewed publications that contained *in vitro* ER TA assay 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.

In *in vitro* ER TA assays, the cell lines used include those that have been transfected with foreign DNA consisting of an ER from human or mouse and a reporter gene that is transcribed as a consequence of a substance binding with the ER, and mammalian cells harboring an endogenous ER in which either a reporter gene is added or cell proliferation is measured as an endpoint. Studies that evaluated the potential ER agonism of a test substance used enzyme activity as an indirect measure of ER-induced transcriptional activation, while ER antagonism studies measured the ability of a test substance to inhibit transcription of a reporter gene (i.e., luciferase or chloramphenicol acetyltransferase in mammalian cell assays, and β-galactosidase in yeast assays). The agonistic potency of a substance in *in vitro* ER TA assays has been determined by comparing the enzyme activity induced by the test substance with that produced by the reference estrogen. Cell proliferation studies reported results as cell number, foci/cm^2, EC_{50} values, cell

growth relative to hormone free control, increase in protein or DNA content, and fold increase in cell proliferation relative to vehicle control. Data from each study was assigned a qualitative response of "positive" or "negative" for the particular assay system, to the extent possible from the information available in the publication.

A variety of yeast and mammalian cell reporter gene systems was used in the studies. The yeast assays used different strains of yeast and sources of ER (e.g., human, mouse, rainbow trout) that were usually stably integrated into the yeast genome. The mammalian cell reporter gene assays used a variety of cell types, including human breast cancer cell lines (MCF-7, MDA-MB-231, T47D, ZR-75); human ovarian carcinoma cells (BG-1); Chinese hamster ovary cells (CHO-K1); monkey kidney cells (COS-1); rat uterine leiomyoma cells (ELT-3); human endometrial cancer cells (HEC-1 and Ishikawa); human embryonal kidney cells (HEK293); human cervical tumor cells (HeLa); and human liver tumor cells (HepG2). The mammalian cell reporter gene systems used various permutations of stable and transient transfections of ER, and ERα and ERβ subtypes from the human and mouse. Different methods were used to assess cell toxicity (e.g., β-galactosidase expression and cell counts and staining). Data and methods were collected also for *in vitro* ER cell proliferation assays.

In vitro ER TA assay data were collected for a total of 698 substances of which 534 substances were tested in reporter gene agonism assays and 174 in reporter gene antagonism assays. A total of 312 substances were tested for agonism and 67 for antagonism in the cell proliferation assays. Only 42 substances were tested in five or more reporter gene agonism assays. The chemical classes that have been tested most extensively are polychlorinated biphenyls, organochlorines, polycyclic aromatic hydrocarbons, phenolic steroids, nonphenolic steroids, phthalates, phenols, and alkylphenols. The most common product classes tested in *in vitro* ER TA assays have been pesticides (including their metabolites and degradation products), pharmaceuticals, chemical intermediates, dielectric fluid components, natural products (including several phytoestrogens), and plasticizers.

Data was generated from 22 *in vitro* yeast ER reporter gene assays involving 13 different strains and from 63 *in vitro* mammalian cell ER reporter gene assays using 12 different mammalian cell

lines. Three cell lines using different transfected ER and reporter plasmids were used to measure cell proliferation.

The quantitative results of the *in vitro* ER TA studies for agonism were most commonly presented in terms of relative activity expressed as the fold induction of enzyme activity produced by the test substance relative to the activity in the untreated controls, as the ratio of the response of the test substance to that of the reference estrogen, or as the concentration of the test substance that produced a certain percent response relative to the reference estrogen. An EC_{50} value (the half-maximal concentration) was provided infrequently. For antagonism studies, the inhibition of reference estrogen-induced enzyme activity by the test substance was measured and sometimes expressed as an IC_{50} value (i.e., the concentration that inhibited the reference estrogen -induced ER transcriptional activation by 50%).

Relatively few of the substances had been tested by more than one investigator in the same *in vitro* ER TA assay or in multiple assays in the same or different laboratories. Furthermore, because the primary focus of many of the studies reviewed in this BRD was on understanding the mechanisms of ER-induced transcriptional activation and not on identifying substances with ER agonist or antagonist activity, much of the published data are of limited value for the analysis of assay performance or reliability.

Based on the limited data available, there is no single *in vitro* ER TA assay that can be concluded to perform better or to be more reliable than any other assay. However, it might be anticipated that mammalian cell assays would be preferred over yeast assays, simply because of the increased ability of test substances to cross the mammalian cell membrane compared to the yeast cell wall. Although the transiently transfected cell lines have some advantages over the stably transfected cell lines in that the level of the ER is higher in the former, the ability to reproducibly transfect the same amount of DNA on a routine basis is difficult. Not all of the laboratories using this technique monitored the transfection efficiency. Taking these factors into consideration, a cell line stably transfected with both the hER expression and the luciferase reporter plasmids (e.g., PALM) or one with an endogenous ER and a stably transfected reporter plasmid (e.g., BG-1) would offer the greatest utility by eliminating the need to continuously

prepare multiple batches of transiently transfected cells.

Formal validation studies should be conducted using appropriate substances covering the range of expected EC_{50}/IC_{50} values to adequately demonstrate the performance characteristics of any *in vitro* ER TA assay recommended as a possible screening test method for ER agonists and antagonists. The role of metabolic systems in activating some substances to ER agonists or antagonists needs to be considered prior to the implementation of future validation studies.

An important step towards acceptance of an *in vitro* ER TA assay into a regulatory screening program is production of high quality data. To achieve this goal, it is recommended that any future pre-validation and validation studies on *in vitro* ER TA 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.

The facilities needed to conduct *in vitro* ER TA assays are widely available, as is the necessary equipment from major suppliers. Although information about the commercial cost of these assays was not available, it can be assumed that the costs for most if not all of the assays are roughly equivalent.

Since there are no published guidelines for conducting *in vitro* ER TA studies, and no formal validation studies have been performed to assess the reliability or performance of such assays, the U.S. EPA requested that minimum procedural standards based on a comparative evaluation of *in vitro* ER TA assays be developed. The minimum procedural standards provided include methods for determining the ability of the reference estrogen to induce transcriptional activation, methods for establishing a stable cell line, the concentration range of the test substance (including the limit dose) to test for agonists and antagonists, the use of negative and positive controls, the number of replicates to use, 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 TA studies will be conducted in such a manner as to allow the results to be understandable and comparable among procedures.

Six *in vitro* ER TA assay protocols developed by experts in the field are provided in **Appendix B**. Inspection of these protocols provides a perspective on how various *in vitro* ER TA assays are conducted by different investigators, and for developing a more general protocol, one that takes into account the recommended minimum procedural standards. Prior to developing that protocol, the submitted protocols need to be evaluated for completeness and adequacy for their intended purpose.

The U.S. EPA requested that a list of recommended test substances be provided for use in validation studies. Testing of substances encompassing a wide range of agonist/antagonist responses are needed to adequately demonstrate the performance characteristics of *in vitro* ER TA test methods recommended as screening assays. A number of factors were considered in developing this list of substances, including the EC_{50} and IC_{50} value of the substance in the assays in which it had been tested. The selected substances were sorted according to whether they were positive, weak positive, or negative in at least one *in vitro* ER TA assay.

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

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1.0 INTRODUCTION AND RATIONALE FOR THE USE OF *IN VITRO* ER TA 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 in 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 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 suggest 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), precocious puberty and 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 (Barlow et al., 1999; Safe, 2000).

In 1996, the U.S. Congress responded to societal concerns by enacting 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 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 proposed 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, and thyroid hormone systems. Tier 2 is comprised of *in vivo* assays 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) assays
- AR binding/TA assays
- 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 assays
- Invertebrate reproduction assay

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;
- 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 measure the biological response to receptor binding (i.e., RNA transcription) and thus, unlike binding assays, can distinguish between an agonist (i.e., a substance that mimics the action of endogenous hormones) and an antagonist (a substance that binds to a receptor without eliciting 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 of an adverse health effect in humans or wildlife.

As part of the validation process for the proposed EDSP assays, the U.S. EPA is supporting an effort by NICEATM 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 submitted 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 the effectiveness of the assays for identifying endocrine-active substances;
- Develop minimum procedural standards for acceptable *in vitro* ER and AR binding and TA assays; and
- Generate a list of substances suitable for use in future validation studies.

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

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

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

1.2.1 Purpose for Using *In Vitro* ER TA Assays

The *in vitro* ER TA assays are designed to identify substances that might interfere with normal estrogen activity *in vivo* by acting as an agonist or antagonist. Unlike receptor binding assays, TA assays can distinguish between these two types of activity. *In vitro* ER TA assays used to evaluate agonism are generally performed by quantifying the induction of a reporter gene product or the stimulation of cell growth in response to activation of the ER by the test substance. *In vitro* ER TA assays that evaluate antagonism measure the ability of a test substance to inhibit the induction of the reporter gene product or the stimulation of cell growth by a reference estrogen. However, a positive response in an *in vitro* ER TA agonist or antagonist assay is not sufficient to predict *in vivo* effects. For this reason, results of the *in vitro* ER TA assays will be used in conjunction with Tier 1 *in vivo* screening assays in a weight-of-evidence approach to prioritize substances for Tier 2 testing.

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

Reporter gene assays provide a relatively simple way to measure whether a substance can activate or inhibit the transcriptional activation of estrogen-regulated genes. The accurate quantitation of the ER-dependent transcriptional activation of endogenous, hormone-dependent genes has been difficult, due largely to the complex signaling networks and transcriptional

controls that are involved in the process. An ER reporter gene assay eliminates these complexities by creating an artificial gene expression system in a host cell. These assays use cellular processes that have been genetically manipulated to allow for the measurement of one specific gene product, typically an enzyme, the production of which is controlled by the ER. Since most cultured cells lack the ER and some of the necessary components of the pathway for ER transcriptional activation, these genes must be inserted into each cell. This is accomplished by transfecting a plasmid containing ER complementary DNA (cDNA) and estrogen-responsive promoters into the host cell, along with the cDNA for a reporter gene, which is linked to an estrogen response element (ERE).

The isolation of the cDNA of the human ER (hER) by Green et al. (1986) permitted the development of *in vitro* assays to measure ER-induced transcriptional activation. In 1988, it was demonstrated that estrogen can bind to the recombinant hER produced in yeasts, and that this interaction of hormone with receptor is capable of directing hormone-dependent activation of genes containing EREs (Metzger et al., 1988). With these characteristics in mind, researchers began to engineer yeast cells by reconstituting a hormone-responsive transcription unit in the cells and by using novel gene fusion technology to produce an active human steroid receptor (McDonnell et al., 1989). Two vectors were constructed. The expression vector contained cDNA of the human ER that was attached to the copper-responsive yeast metallothionein (CUP1) promoter to drive the synthesis of receptor messenger RNA (mRNA). Initiation in this vector was from the natural start codon of the receptor. The CUP1 promoter is tightly regulated by copper ions, thus permitting controlled expression of the receptor in the yeast cell. The reporter plasmid contained two copies of the vitellogenin response element upstream of the yeast iso-1-cytochrome c promoter fused to the β-galactosidase gene.

Since the initial report on the engineering of these yeast strains, other yeast transformants suitable for use in *in vitro* ER TA assays have been constructed. The transformed yeast strains produced by Routledge and Sumpter (1996, 1997) have been disseminated to a number of laboratories for measurement of ER-induced transcriptional activation. These yeasts have been transformed with the hER expression plasmid containing the CUP1 promoter and a reporter plasmid containing the frog vitellogenin response element and the 3-phosphoglycerate kinase

(pgk) promoter. The level of expression of the ER is lower in yeasts than in mammalian cells; this may be due to the absence of certain co-activators normally active in mammalian cells during ER-induced transcriptional activation.

During the last few years, mammalian cell lines have been increasingly used to measure ER-induced transcriptional activation. Cell lines used most frequently include HEK293, HEC-1, HeLa, HepG2, and MCF-7; those used less frequently include Chinese hamster ovary (CHO), BG-1, COS-1, ELT3, MDA-MB-231, T47D, and Ishikawa. The majority of expression vectors transfected into these cell lines contain the hERα or the hERβ. Used less frequently have been expression vectors containing the mouse or rainbow trout ER (mER and rtER, respectively) and the "def" or ligand binding domains of hER and mERβ (**Appendix D**). Both transient and stable transfection techniques have been used.

In the production of transiently transfected cell lines that lack an endogenous ER, two vectors are introduced simultaneously into the cells. One is an ER expression vector containing ER cDNA, while the other is a reporter vector containing a gene for an enzyme linked to an upstream promoter and two to four EREs. Chloramphenicol acetyltranserase (CAT) was originally selected as the reporter gene because it was absent in mammalian cells and because the assay was considered relatively sensitive (Gorman et al., 1982). More recently, the easier to use and more sensitive luciferase assay has been the reporter of choice. The most commonly used response elements are derived from the frog vitellogenin response element while the promoter is from the thymidine kinase, C3, or pS2 genes. In some studies, the cells used were stably transfected with the ER while the reporter vector has been almost always transiently transfected into the cells of interest. In cell lines that contain an endogenous ER (e.g., MCF-7, BG-1 and T47D), only the reporter plasmid needs to be transfected.

Several transfection procedures have been used, including calcium phosphate precipitation, viral transduction, electroporation, and the use of commercial transfection reagents (e.g., FuGene™, LipoFect™ AMINE). Each of these agents appear to be efficacious to different extents in different cell lines, but no formal evaluation of these different techniques has been conducted.

Cell proliferation has also been used as an indicator of estrogen-induced transcriptional activation. The MCF-7 cell line, which was established from a metastatic mammary carcinoma and which contains an endogenous ER, is the cell line used most frequently for this purpose but other human-derived cell lines that have an endogenous ER (e.g., ZR-75 and T47D) have been used also.

Data analysis approaches reported in the literature have varied from a visual inspection of the data to more formal statistical approaches that use either one- or two-way analysis of variance (ANOVA) (with main effects being treatment or replicates and treatment, respectively). Most of the yeast-based assays reported their results in Miller Units (see **Section 2.3.2**), which can be used to calculate EC_{50} values, the concentration of an agonist that produces 50% of the maximal reporter gene response. From these values, potency ratios (EC_{50} test substance/EC_{50} 17β-estradiol or relative potency (i.e., percent of maximal reference estrogen response) have been derived. For mammalian cell studies, the results for agonists have been reported as fold induction or increase in enzyme activity compared to the concurrent vehicle control, as EC_{50} values, or as relative potency ratios based on the response for the reference estrogen. For mammalian cell antagonist assays, the response elicited by the test substance in combination with the reference estrogen is compared to the response induced by the reference estrogen alone. The resulting data have been expressed as a relative fold induction in response or as IC_{50} values (i.e., the concentration of the test substance inhibiting the reference estrogen response by 50%). The EC_{50} values (for agonist assays) or IC_{50} values (for antagonist assays) have been calculated using various curve-fitting programs. One curve fitting approach used is based on a logistic dose response model where the asymptotic minimum and maximum response, the dose that is halfway between the minimum and maximum, and the slope of the line tangent to the logistic curve at this mid-point, is determined (Gaido et al., 1997).

The *in vitro* ER TA reporter gene assays produce measures of enzyme activity. The values obtained depend on a number of factors, such as the specific assay system used, the binding affinity of the test substance for the ER, the ER concentration, and the experimental conditions (e.g., cell type, transfection efficiency, pH, exposure duration). Because different investigators have reported their data in a variety of formats, comparison of data for the same substance tested

in different assays has proven to be difficult. The EC_{50} values that have been reported cover approximately seven orders of magnitude. However, there is no current guidance as to which levels of activity are biologically meaningful.

The primary focus of the initial *in vitro* ER TA studies conducted prior to the mid-1990s was on mechanisms. However, by the year 2000, the majority of *in vitro* ER TA assay-related publications have focused on the testing of industrial chemicals and environmental contaminants. Currently, there are no standardized *in vitro* ER TA assays for the routine testing of substances for ER agonist or antagonist activity. The *in vitro* ER TA assays, as currently performed, are described in detail in **Section 2.0**.

1.2.3 Mechanistic Basis of *In Vitro* ER TA Assays

Transcriptional activation is one step in a series of events that is used to control gene expression in an estrogen responsive cell. The ER is the primary receptor for endogenous estrogens that enter the cell from the bloodstream to initiate the transcription of mRNA and ultimately protein synthesis. The interaction of estrogens with the ER in a cell initiates a cascade of events. Upon ligand binding, the ER undergoes a conformational change that allows dissociation of co-repressor proteins from the ER and the recruitment of co-activator proteins. The ligand-bound ER complex dimerizes and binds, in the presence of co-activator molecules, to an ERE located upstream from the genes under estrogen control. 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, and adult homeostasis.

The ER, a transcriptional regulatory protein belonging to the nuclear hormone receptor superfamily, is involved in steroid hormone signaling, functioning as a ligand-dependent transcriptional activator. The ER protein plays a major role in controlling the transcriptional activation and/or repression of estrogen-responsive genes. The ER is localized in the soluble nuclear fraction of estrogen target cells. The hER gene was cloned and sequenced by Green et al. (1988); the protein contains 595 amino acids, organized into several discrete domains that are involved in transcriptional activation. These include an A/B domain in the *N*-terminal region

that contains an activation function (AF1); a DNA-binding domain in the middle of the molecule; a hinge region; and a ligand binding domain that contains a second activation function (AF2) domain located in the *C*-terminal region of the protein (Kumar and Thompson, 1999).

The ER is associated with heat shock proteins in the cell and a transcriptionally active form is generated after 17β-estradiol binds to the ligand binding domain in the *C*-terminal part of the molecule. The centrally located, highly conserved DNA binding domain mediates the interaction of the activated ER with the HREs on the DNA. Sequences within the *C*-and *N*-terminal domains interact with transcriptional intermediary factors (TIF) found in the cell. Transcriptional activation of estrogen responsive genes occurs following the binding of the ER-ligand complex to response elements on the DNA. Ligand-binding transcriptional activation by the ER also involves the recruitment of a number of transcriptional mediating proteins as well as the AF2 domain on the ER. The AF2 domain is required for transcriptional activation and mutations in this region of the ER result in a loss of transcriptional activation capacity (Tasset et al., 1990).

The current hypothesis for ER-mediated endocrine disruption is that xenobiotic substances that are similar in structure to 17β-estradiol, the natural ligand for the ER, may mimic or block its activity. The former action would produce an estrogen-like effect while the latter would interfere with normal, physiological, estrogen-mediated processes. In some cases, antagonists might not bind directly to the ER but rather inhibit the interaction of an activated receptor with other factors required for transcriptional activation (Pham et al., 1992).

Since transcriptional activation cannot occur unless an agonist first binds to the ER, factors that affect binding have an impact on this process. These factors include:

- *Affinity for the ER.* This affinity depends on the rates of the association and disassociation of the ligand with the receptor. However, little is known of these rates in the artificial yeast and mammalian cell systems used to study ER-induced transcriptional activation.
- *Systemic half-life of the ligand.* The *in vivo* half-life will depend on the rate of metabolism of the substance to an active intermediate or to an inactive product, and to the clearance of the ligand and its metabolites from the organism. The metabolic capacity of the cells used for *in vitro* ER TA assays is generally unknown but probably limited.

1.2.4 Relationship of Mechanisms of Action in the *In Vitro* ER TA Assay Compared to the Species of Interest

The ER is highly conserved among vertebrate species, and substances that activate or inhibit ER-induced transcriptional activation in one species are expected to have the same activity in other vertebrate species. However, because of differences in the types and rates of the associated substances that interact with the receptor-ligand complex, the relative activity of a substance may vary in different tissues of the same animal, and among different species.

Due to a lack of information on interspecies comparisons, the present working hypothesis is that estrogen-induced biological effects in one vertebrate species are expected to occur in other species. This hypothesis is the basis for the use of *in vitro* ER TA assays as a general screen for estrogenic effects. The most widely used *in vitro* assay systems use hER in human or yeast cells. Substances that bind the ER in these cells and initiate or inhibit transcriptional activation of ER-responsive genes are presumed to be capable of producing estrogenic effects in multiple species. However, studies to support this working hypothesis are yet to be conducted.

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

In vitro ER TA assays are proposed as components of the EDSP Tier 1 screening battery. The Tier 1 screening battery is comprised of multiple *in vitro* and *in vivo* assays designed to assess both receptor- and non-receptor-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* ER TA Assays

The FQPA requires the U.S. EPA to 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 *in vitro* ER TA assays described in this BRD, relevance is restricted to how well an assay identifies substances that are capable, *in vitro*, of activating or inhibiting transcription of estrogen-inducible genes. The reliability of an assay is defined as its reproducibility within and among laboratories, and 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 evaluates the relevant data and information about the assay, including its mechanistic basis, proposed uses, reliability, and performance characteristics (ICCVAM, 1997). This BRD summarizes the available information on the various types of *in vitro* ER TA assays that have been commonly used to characterize substances as potential endocrine disruptors. Where appropriate data are available, the qualitative and quantitative performances 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., mammalian cell-based assay or yeast-based assay using stably or transiently transfected ER and reporter genes) have 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 for current and future *in vitro* ER TA assays.

1.3.2 Where Can *In Vitro* ER TA 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 as validated assays. The *in vitro* ER TA assays are intended, along with other *in vitro* and *in vivo* tests, to be a component of the proposed EDSP Tier 1 screening battery for identifying endocrine disruptors.

1.3.3 Similarities and Differences with Currently Used Methods

The *in vitro* assessment of ER-induced transcriptional activation is not an endpoint currently required for regulatory decision-making. However, there are a number of *in vitro* assays available for assessing the ability of test substances to induce ER-dependent transcriptional activation. These assays are based on the same general principles, but often use different cell lines, ER sources, and protocols.

The most frequently used *in vitro* ER TA assays use mammalian cell lines that are transiently or stably transfected with vectors encoding hER and a reporter enzyme, typically luciferase. To test the potential agonism of a substance, transcriptional activation is measured as the amount of reporter gene product (e.g., luciferase activity) induced by the test substance. Antagonism of a test substance is quantified by measuring the reduction of enzyme activity that occurs when the test substance and the reference estrogen are incubated together.

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

The *in vitro* ER TA assays are proposed as a component of the EDSP Tier 1 screening battery that also includes androgen, estrogen, and thyroid receptor-binding assays, *in vitro* AR TA assays, and *in vivo* assays for endocrine effects in rodents, amphibians, and fish. EDSTAC recognized that TA assays, because they also measure the consequences of binding, provide more information than binding assays. However, the limited databases at that time did not allow a determination of whether assays that measured binding or TA or both 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 screening 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 the TA process, and might produce results that are not biologically meaningful *in vivo* as a result of limited absorption and distribution, or rapid 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*, or to *in vivo* endocrine-related effects that are mediated by mechanisms not addressed by the *in vitro* assays.

A positive result in an *in vitro* ER TA assay (or in any Tier 1 screening assay) is not, in itself, 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 subjected 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 the *In Vitro* ER TA Assay and/or Limits of the *In Vitro* ER TA Assay

The range of substances amenable to testing in *in vitro* ER TA assays has yet to be determined and will depend on the outcomes of an independent peer review of the assays considered in this BRD and any future validation studies. The *in vitro* ER TA 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) under the following three 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; P.L. 105-115, 1997) 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 TA BRD

The *in vitro* ER TA assay data summarized in this BRD are based on information found in the peer-reviewed scientific literature and in submitted reports. An online literature search was conducted for entries in MEDLINE, CANCERLIT, TOXLINE, AGRICOLA, NIOSHTIC, EMBASE, CABA, BIOSIS, and LifeSci that reported on the *in vitro* testing of substances for 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 TA assays were sought. The search strategy involved the combining of "*vitro*" with alternative terms for estrogens, receptors, binding, transcription, activation, and testing. Each database record included authors, bibliographic citation, and indexing terms. Most records also included abstracts.

Two hundred fifty-eight articles relating to *in vitro* ER TA studies were identified. Abstracts of these articles were reviewed and full text copies of articles judged to be relevant were obtained and a bibliographic database of the literature citations established. Scanning of the literature using *Current Contents* and the British Lending Library's *Table of Contents* continued through the writing of the BRD, and recently published articles were added to the database as they became available. Identification of ER TA-related publications for data extraction ended on January 25, 2002.

The most relevant reports were those containing data on substances that had 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. Data were not extracted from reports of studies that tested obscure compounds, such as structural or positional isomers of known binding agents, if the compounds had not been tested in a commonly used protocol. However, data were extracted from reports of studies using unique procedures if the study

included substances that had been tested in one of the more commonly used assays. Of the publications identified, 86 contained data that have been abstracted and included in this BRD. A large proportion of these publications included data from more than one assay (e.g., different cell types, different reporter constructs). In addition, this BRD contains data from two unpublished reports that were submitted in response to the FR notice requesting *in vitro* ER TA assay data.

2.0 METHODS FOR *IN VITRO* ER TA ASSAYS

2.1 Introduction

A number of different *in vitro* methods have been used to measure ER-induced transcriptional activation. However, currently, there are no generally accepted standardized methods for these assays. The *in vitro* assays used to identify ER agonists and antagonists can be classified into three broad groups: reporter gene assays using yeast cells; reporter gene assays using mammalian cells, and cell proliferation assays using mammalian cells. Most, but not all, of the mammalian cell lines used in the reporter gene assays lack an endogenous ER and must therefore be transiently or stably transfected with a plasmid carrying the ER and promoter genes. Yeast cells do not have an endogenous ER and must be transformed using an ER-containing plasmid. The cell lines used in cell proliferation assays contain an endogenous ER eliminating the need for transfection. The sensitivity and/or responsiveness of each cell line are determined by the characteristics of the cells themselves, the constructs used, and either by the efficiency of the transient transfection or by the concentration of the stably transfected/endogenous ER.

Many of the published *in vitro* ER TA studies were conducted specifically to test substances for ER agonist or antagonist activity; in addition, some studies have been conducted to investigate the process of ER-induced TA or to identify structure-activity relationships (SAR). Data from a total of 86 peer-reviewed publications and two submitted reports containing unpublished data have been extracted for this BRD. The publications and reports describe studies using a number of undefined genera of yeast (most likely *Saccharomyces cerevisiae*), various strains of *S. cerevisiae,* and a number of different mammalian cell lines. A list of the yeast strains and mammalian cell lines used in these studies are summarized in **Tables 2-1 to 2-3**. Information provided includes, where specified, the designation for the mammalian cell line or yeast strain, the ER source, the ER subtype, the investigators' designation(s) for the plasmid(s), the reference, and the designation for the assay used in this BRD. The BRD assay designation includes the name of the cell line or yeast strain, the source of the ER, the reporter gene product or endpoint assessed, and the product of any other plasmid. In addition, the tables indicate whether any vector used was stably or transiently transfected into the cell line or yeast.

Table 2-1 *In Vitro* Yeast ER TA Reporter Gene Studies (Arranged by Strain)

Species and Strain	Plasmids Transfected	BRD Designation	Reference
S. cerevisiae	hERα + ERE-LacZ	Yeast(*S.cer.*) hERα(S)+β-gal(S)	De Boever et al. (2001)
	hERα + PPK-ERE-LacZ	Yeast(*S.cer.*) hERα(S)+β-gal(S)	Elsby et al. (2001)
	hERα + ppK-vit2ERE-LacZ	Yeast(*S.cer.*) hERα(S)+β-gal(S)	Beresford et al. (2000); Miller et al. (2001)
	hER + ppK-vit2ERE-LacZ	Yeast(*S.cer.*) hER(S)+β-gal(S)	Routledge and Sumpter (1996; 1997); Harris et al. (1997); Odum et al. (1997); Moffat et al. (2001); Rajapakse et al. (2001); Yoshihara et al. (2001); Vinggaard et al. (1999; 2000);
S. cerevisiae 190	hERα + LacZ	Yeast(*S.cer.*190) hERα(E)+β-gal(S)	Morito et al. (2001a,b)
	hERβ + LacZ	Yeast(*S.cer.*190) hERβ(E)+β-gal(S)	Morito et al. (2001a)
S. cerevisiae 939	hER + ERE-CYC-1-LacZ	Yeast(*S.cer.*939) hER(S)+β-gal(S)	Chen et al. (1997)
*S. cerevisiae** 188R1	hER-CUP1 + A2ERE.cyc1.LacZ	Yeast(*S.cer.*188R1) hER(S)+β-gal(S)	Graumann et al. (1999)
S. cerevisiae BJ2168	mER + vitERE-LacZ	Yeast(*S.cer.*BJ2168) mER(S)+β-gal(S)	Ramamoorthy et al. (1997a)
S. cerevisiae BJ2407	hER + YRPE2-LacZ	Yeast(*S.cer.*BJ2407) hER(S)+β-gal(S)	Klotz et al. (1996)
	hER + vERE-CYC1-LacZ	Yeast(*S.cer.*BJ2407) hER(S)+β-gal(S)	Ramamoorthy et al. (1997a)
	hER + ERE-LacZ	Yeast(*S.cer.*BJ2407) hER(S)+β-gal(S)	Arnold et al. (1996)
S. cerevisiae BJ3505	hER + vitERE-LacZ	Yeast(*S.cer.*BJ3505) hER(S)+β-gal(S)	Ramamoorthy et al. (1997b)
	CUP1MET + 2FR.vit-iso1-cytC-LacZ	Yeast(*S.cer.*BJ3505) hER(S)+β-gal(S)	Coldham et al. (1997)
	hER-CUP1-MET + ERE-LacZ	Yeast(*S.cer.*BJ305) hER(S)+β-gal(S)	Gaido et al. (1997)
S. cerevisiae BJ-ECZ	hER + ERE2-CYC-1-LacZ	Yeast(*S.cer.*BJ-ECZ) hER(S)+β-gal(S)	Le Guevel and Pakdel (2001)
	rtER + ERE2-CYC1-LacZ	Yeast(*S.cer.*BJ-ECZ) rtER(S)+β-gal(S)	Petit et al. (1997, 1999); Le Guevel and Pakdel (2001)
*S. cerevisiae** CYT10-5d	hER + ERE-LacZ	Yeast(*S.cer.*CYT10-5d) hER(S)+β-gal(S)	Chen et al. (1997)

Species and Strain	Plasmids Transfected	BRD Designation	Reference
*S. cerevisiae** ER	hER + LacZ	Yeast(*S.cer*.ER) hER(S)+β-gal(S)	Tran et al. (1996)
*S. cerevisiae** ER179C	hER + LacZ	Yeast(*S.cer*.ER179C) hER(S)+β-gal(S)	Tran et al. (1996)
S. cerevisiae PL3	Yeast PL3-hERαdef + ERE-URA	Yeast(*S.cer*.-PL3) hERαdef(S)+β-gal(S)	Connor et al. (1996); Zacharewski et al. (1998)
S. cerevisiae YRG-2	hERα + ERE-CYC-1-LacZ	Yeast(*S.cer*.YRG-2) hERα(S)+β-gal(S)	Lascombe et al. (2000)

* Species name was not provided in publication, but is likely *S. cerevisiae.*
Abbreviations: β-gal = β-Galactosidase, CAT = Chloramphenicol acetyl transferase; (E) = Endogenous, ER = Estrogen receptor, ERE = Estrogen response element, h = Human, Luc = Luciferase, m = Mouse, r = Rat, rt = rainbow trout, (S) = Stably transfected, (T) = Transiently transfected, vit = Vitellogenin.

Table 2-2 *In Vitro* Mammalian Cell ER TA Reporter Gene Studies (Arranged by Cell Line)

Cell Line	Plasmids Transfected	BRD Designation	Reference
BG-1	hER + ERE-MMTV-Luc	BG1 hER(E)+Luc(S)	Xenobiotic Detection Systems (2001)
	hER + MMTV-Luc	BG1 hER(E)+Luc(T)	Rogers and Denison (2000)
CHO-K1	hERα + ERE-hs-Luc	CHO-K1 hERα(S)+Luc(S)	Otsuka Pharmaceutical (2001)
	hERα + ERE-tk-Luc	CHO-K1 hERα(S)+Luc(T)	Otsuka Pharmaceutical (2001)
COS-1	mERα + vitA2EREB-Luc	COS-1 hERα(T)+Luc(T)	Tremblay et al. (1998)
	mERα + vitA2ERETK-Luc	COS-1 mERα+Luc	Tremblay et al. (1998)
	mERβ + vitA2EREB-Luc	COS-1 hERβ(T)+Luc(T)	Tremblay et al. (1998)
	mERβ + vitA2ERETK-Luc	COS-1 mERβ+Luc	Tremblay et al. (1998)
ELT-3	hER + vitERE-tk-Luc6a + CMV- LacZ	ELT-3 hER(T)+Luc(T)+β-gal(T)	Hodges et al. (2000)
HEC-1	hERα + C3-Ti-Luc	HEC-1 hERα(T)+Luc(T)+β-gal(T)	Sun et al. (1999)
	hERα + ERE-pS2-CAT	HEC-1 hERα(T)+CAT(T)+β-gal(T)	Meyers et al. (1999); Sun et al. (1999)
	hERα + ERE-pS2-CAT + CMVβ-gal	HEC-1 hERα (T)+CAT(T)+β-gal(T)	Kraichely et al. (2000)
	hERβ + C3-Ti-Luc	HEC-1 hERβ(T)+Luc(T)+β-gal(T)	Sun et al. (1999)
	hERβ + ERE-pS2-CAT	HEC-1 hERβ(T)+CAT(T)+β-gal(T)	Meyers et al. (1999); Sun et al. (1999)
	hERβ + ERE-pS2-CAT + CMVβ-gal	HEC-1 hERβ(T)+CAT(T)+β-gal(T)	Kraichely et al. (2000)
HEK293	hERα + 3ERE-tata-Luc	HEK293 hERα(S)+Luc(S)	Meerts et al. (2001)
	hERα + 3ERE-tata-Luc	HEK293 hERα(S)+Luc(T)	Seinen et al. (1999)

Cell Line	Plasmids Transfected	BRD Designation	Reference
	hERα + 3ERE-tata-Luc	HEK293 hERα(T)+Luc(T)	Seinen et al. (1999)
	hERα + ERE-Luc	HEK293 hERα(T)+Luc(T)	Collins-Burow et al. (2000)
	hERα + ERE-TATA-Luc + SV2-LacZ	HEK293 hERα(T)+Luc(T)+β-gal(T)	Kuiper et al. (1998)
	hERβ + 3ERE-tata-Luc	HEK293 hERβ(S)+Luc(S)	Meerts et al. (2001)
	hERβ + 3ERE-tata-Luc	HEK293 hERβ(S)+Luc(T)	Seinen et al. (1999)
	hERβ + 3ERE-tata-Luc	HEK293 hERβ(T)+Luc(T)	Seinen et al. (1999)
	hERβ + ERE-Luc	HEK293 hERβ(T)+Luc(T)	Collins-Burow et al. (2000)
	hERβ + ERE-TATA-Luc + SV2-LacZ	HEK293 hERβ(T)+Luc(T)+ β-gal(T)	Kuiper et al. (1998)
HeLa	GAL4HEG0 + 17m5-G-Luc	HeLa hERαdef(S)+Luc(S)	Connor et al. (1997); Moore et al. (1997); Zacharewski et al. (1998)
	hER + p17m5-G-Luc + AG60.neo	HeLa hER(S)+Luc(S)	Balaguer et al. (1996)
	hER + vitA2ERE-TK-CAT	HeLa hER(T)+CAT(T)	Miksicek (1993; 1994)
	hERα + ERE-Luc	HeLa hERα(T)+Luc(T)+β-gal(T)	Gaido et al. (1998)
	hERα + TK-XvitERE-Luc	HeLa hERα(T)+Luc(T)	Sumida et al. (2001)
	hERα + XvitERE-tk-Luc	HeLa hERα(T)+Luc(T)	Tarumi et al. (2000)
	hERβ + ERE-Luc	HeLa hERβ(T)+Luc(T)+β-gal(T)	Gaido et al. (1999)
	mER + ERE81CAT	HeLa mER(T)+CAT(T)	Makela et al. (1994); Shelby et al. (1996); Garner et al. (1999)
HepG2	hER + C3-Luc + β-gal	HepG2 hER(T)+Luc(T)+β-gal(T)	Ramamoorthy et al. (1997b)
	hERα + C3-Luc + CMV-β-gal	HepG2 hERα(T)+Luc(T)+β-gal(T)	Gaido et al. (1999; 2000)
	hERα + ERE-C3-Luc	HepG2 hERα(T)+Luc(T)+β-gal(T)	Gould et al. (1998)
	hERβ + C3-Luc + CMV-β-gal	HepG2 hERβ(T)+Luc(T)+β-gal(T)	Gaido et al. (1999, 2000)
	rERα + C3-Luc + CMV-β-gal	HepG2 rERα(T)+Luc(T)+β-gal(T)	Gaido et al. (1999)
	rERβ + C3-Luc + CMV-β-gal	HepG2 rERβ+Luc(T)+β-gal(T)	Gaido et al. (1999)
Ishikawa	hER + vitERE-Luc + CMV-β-gal	Ishikawa hER(T)+Luc(T)+β-gal(T)	Klotz et al. (1996)
MDA-MB-231	hER + CKB-Luc	MDA-MB-231 hER(T)+Luc(T)	Ramamoorthy et al. (1997b)
	hERα + ERE-Luc	MDA-MB-231 hERα(T)+Luc(T)	Bonefeld-Jørgensen et al. (2001)
MCF-7	GAL4HEG0 + 17m5-G-Luc	MCF-7 hERαdef(T)+Luc(T)	Connor et al. (1996)
	GAL4HEG0 + 17m5-G-Luc + CH110 (β-gal)	MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Charles et al. (2000a,b)
	Gal4-HEG0 + 175m5-G-Luc + CMV-lacZ	MCF-7 hERαdef (T)+Luc(T)+β-gal(T)	Zacharewski et al. (1998); Fertuck et al. (2001a,b)
	GAL4HEG0 + 17m5-G-Luc + CMV (β-gal)	MCF-7 hERαdef (T)+Luc(T)+β-gal(T)	Clemons et al. (1998)
	GAL4-HEG0 + 17m5-G-Luc + lacZ	MCF-7 hERαdef (T)+Luc(T)+β-gal(T)	Fielden et al. (1997)
	GAL4-hERαdef + 17m5-G-Luc + CMV-lacZ	MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Matthews et al. (2001)

Cell Line	Plasmids Transfected	BRD Designation	Reference
	GAL4-hERβdef + 17m5-G-Luc + CMV-lacZ	MCF-7 hERβdef(T)+Luc(T)+β-gal(T)	Matthews et al. (2001)
	GAL4-mERβdef + 17m5-G-Luc + CMV-lacZ	MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	Fertuck et al. (2001a,b)
	hER + CKB-CAT	MCF-7 hER(T)+CAT(T)	Ramamoorthy et al. (1997a,b)
	hER + ERE + Luc	MCF-7(MELN41)-hER(E)+Luc(S)	Lascombe et al. (2000)
	hER + (ERE)3-SV40-Luc + CMV	MCF7 hER(E)+Luc(T)	Yoshihara et al. (2001)
	hER + ERE-tk-CAT + ON249(β-gal)	MCF-7 hER(E)+CAT(T)+β-gal(T)	Bonefeld-Jørgensen et al. (2001)
	hER + ERE2-Luc + CMV(β-gal)	MCF-7 hERαdef(E)+Luc(T)+β-gal(T)	Klotz et al. (1996)
	hER + ERE-tk-Luc + J7lacZ	MCF-7 hER(E)+Luc(T)+β-gal(T)	Jobling et al. (1995)
	hER + GV-tk-vEREx5-Luc	MCF-7 hER(E)+Luc(T)	Sumida et al. (2001)
	hER + Luc	MCF-7 hER(T)+Luc(T)	Ramamoorthy et al. (1997b)
	hER + Vit-CAT	MCF-7 hER(T)+CAT(T)	Connor et al. (1997)
	hER + Vit-tk-Luc	MCF-7 hER(E)+Luc(S)	Kramer et al. (1997)
	hER + vitERE2-Luc + CMV(β-gal)	MCF-7(M) hER(E)+Luc(T)+β-gal(T)	Collins-Burow et al. (2000)
T47D	hER + CAT	T47D hER(E)+CAT(T)	Nakagawa and Suzuki (2001)
	hER + EREtata-Luc	T47D hER(E)+Luc(S)	Legler et al. (1999); Meerts et al. (2001); Hoogenboom et al. (2001)

Abbreviations: β-gal = β-Galactosidase, CAT = Chloramphenicol acetyl transferase; (E) = Endogenous, ER = Estrogen receptor, ERE = Estrogen response element, h = Human, Luc = Luciferase, m = Mouse, r = Rat, (S) = Stably transfected; (T) = Transiently transfected, vit = Vitellogenin.

Table 2-3 *In Vitro* Mammalian Cell ER TA Cell Proliferation Studies (Arranged by Cell Line)

Cell Line	BRD Designation	Reference
Ishikawa	Ishikawa hER(E) + CP	Le Guevel and Pakdel (2001)
MCF-7	MCF-7 hER(E) + CP	Miksicek (1993); Makela et al. (1994); Soto et al. (1994);Soto et al. (1995); Dodge et al. (1996); Mellanen et al. (1996); Fielden et al. (1997); Harris et al. (1997); Ichikawa et al. (1997); Moore et al. (1997); Ramamoorthy et al. (1997a); Jones et al. (1998); Korner et al. (1998); Go et al. (1999); Miodini et al. (1999); Bonefeld-Jørgensen et al. (2001); Morito et al. (2001a); Nakagawa and Suzuki (2001); Otsuka Pharmaceutical (2001); Payne et al. (2001)
MCF-7 [focus assay]	MCF-7 hER(E) + CP(F)	Gierthy et al. (1997); Arcaro et al. (1998); Tamir et al. (2000)
MCF-7(Bos)	MCF-7(Bos) hER(E) + CP	Schlumpf et al. (2001)
MCF-7(BUS)	MCF-7(BUS) hER(E) + CP	Schafer et al. (1999)
MCF-7(E3)	MCF-7(E3) hER(E) + CP	Wiese et al. (1997); Vinggaard et al. (1999)
MCF-7(M)	MCF-7(M) hER(E) + CP	Collins-Burow et al. (2000)
T47D	T47D hER(E) + CP	Makela et al. (1994); Mellanen et al. (1996); Schafer et al. (1999); Tamir et al. (2000)
ZR-75	ZR-75 hER(E) + CP	Jobling et al. (1995); Harris et al. (1997)
ZR-75-1	ZR-75-1 hER(E) + CP	Schafer et al. (1999)

Abbreviations: CP = Cell proliferation; (E) = Endogenous; ER = Estrogen receptor, (F) = foci; h = Human.

The ER used in the majority of reported *in vitro* ER TA studies was human in origin; a small number of studies used the ER derived from mouse or rainbow trout. As discussed in the ER Binding BRD, two human ER proteins have been isolated. These two proteins, known as ERα and ERβ, are found in different proportions in various human and mammalian tissues, and have different capacities for binding substances of certain chemical classes, particularly

phytoestrogens (Kuiper at al., 1997). Both receptors have been used in *in vitro* ER TA assays. ER-induced transcriptional activation is measured in reporter gene assays by following the production of an enzyme whose synthesis and expression is controlled by an ERE. The reporter plasmid typically contains an ERE which controls the expression of a reporter gene, usually luciferase (*Luc*), chloramphenicol acetyl transferase (*CAT*) or, in yeast, β-galactosidase (β-*gal*). Since the sequence of the ERE is contained within the frog vitellogenin gene, this has frequently been used as the ERE source for these assays. Some *in vitro* ER TA assays use cells that have been stably transfected with the ER or with the ER and the reporter gene vectors.

Regardless of whether transient or stably transfected cells are used in the assays, test substances that enter the cells interact with the ER, which becomes activated by a change in its conformation. The activated ER then binds with soluble cell factors, and the resulting complex binds to the ER response elements on the reporter plasmid. This binding initiates the expression of the reporter gene and the production of its associated enzyme. An appropriate substrate in the incubation mixture is metabolized by the newly synthesized enzyme, resulting in the production of an easily detected product. The majority of *in vitro* ER TA studies using mammalian cell lines have used luciferase to assess transcriptional activation because the assay is more rapid, more sensitive, and easier to perform than CAT-based assays. Also, in contrast to the luciferase-based assay, CAT assays require a radiolabeled substrate (either chloramphenicol or acetyl-CoA). In an alternative approach, binding of an appropriate substance to the endogenous ER stimulates cell division in an estrogen responsive cell line.

The cellular level of the ER, which affects the sensitivity of the assay, is usually two to five fold higher in cell lines transiently transfected with the ER compared to cell lines with an endogenous or stably transfected ER. However, the major disadvantage of using transiently transfected cell lines is the uncertainty of the efficiency of transfection, and hence the repeatability of the assay. An approach to monitor the efficiency of transfection is based on the transfection of a plasmid carrying a gene (typically β-*gal*) that codes for a protein that is produced constitutively into the cell line. The level of this enzyme in transfected cells is used as a measure of the transfection efficiency.

Cytotoxicity can be a complicating factor in *in vitro* ER TA assays, particularly when antagonism is being assessed. The absence of or a decrease in the ER-induced TA response might be the result of cell toxicity rather than reflecting the ability of the test substance to interact with the ER. Cell toxicity can be corrected for by performing a parallel cytotoxicity experiment or by measuring the product of a constitutively active gene transfected into the cell on a separate plasmid. Some of the mammalian cell lines transfected with *luc* or *CAT* reporter constructs have also been transfected with a plasmid coding for β-*gal*. The synthesis of β-galactosidase is independent of a receptor-mediated effect, and a comparison of its level in treated versus control cells can be used as a measure of treatment-related cell toxicity.

In studies to measure agonism, the ER-containing cells are treated with a test substance, and the induction of luciferase, CAT, or β-galactosidase determined. A number of measures have been used to assess whether the test substance induces ER-dependent transcriptional activation. These measures include the relative enzyme activity, usually expressed as the amount of the test substance that elicits a specific response compared to the reference estrogen, the EC_{50} of the substance, or a qualitative assessment of "positive," "negative", or "weak". Relative potencies have been presented also. These are determined by dividing the concentration of a test substance producing a half maximal response by the concentration of 17β-estradiol producing an equivalent response. When cell proliferation was used as the end point, a qualitative response measure was usually provided, but in some cases an EC_{50} or a fold increase compared to the reference estrogen was determined. In some studies, the parameter measured is determined graphically; in others a more complicated procedure using regression analysis has been used. In publications in which numerical data were not provided, values for the EC_{50} or for a relative activity versus the reference estrogen were estimated, if possible. These estimated values are italicized in **Appendix D**.

In the studies conducted to determine antagonism, the cells were treated simultaneously with the test substance and the reference estrogen, and the ability of the test substance to inhibit reference estrogen-induced TA measured. Qualitative results were usually provided, but in a few cases a relative value was presented.

In addition to mammalian cells, various strains of *S. cerevisiae* and other undefined yeast strains (probably *S. cerevisiae*) have been used to assess the ability of substances to act as ER agonists or antagonists. Since yeast cells do not contain an ER, the DNA sequence for the ER is transfected into the cells (Routledge and Sumpter, 1996). Usually, both the ER and reporter gene plasmids have been transfected simultaneously into the cells, and clones harboring both plasmids selected for simultaneously. The production of β-galactosidase has been used as the measure of ER-induced TA. The most commonly used expression plasmid contains the ER and CUP1-MET promoter, while the reporter plasmid contains the ERE (frequently the vitellogenin A2 ERE) and the iso-1-cytochrome c (CYC1) promoter in a *LacZ* fusion vector. Expression of the reporter gene results in the formation of β-galactosidase, which is usually measured following lysis of the cells, although a few investigators have measured enzyme activity in the medium. β-Galactosidase metabolizes a chromogenic galactopyranoside to a chromogenic agent that can be measured spectrophotometrically. For the yeast assays, the activity of β-galactosidase versus the log of the concentration of the test substance is represented graphically. Fold induction of β -galactosidase has also been presented.

Because there are no "consensus" cell lines, vectors, or specific treatment protocols for *in vitro* ER TA studies, the following sections describe general protocols for agonism and antagonism studies using mammalian and yeast cells transfected with both an ER and a reporter gene, cells containing an endogenous ER that are transfected with a reporter gene, and mammalian cells that use growth as an endpoint.

2.2 *In Vitro* Mammalian Cell ER TA Reporter Gene Assays

2.2.1 Expression and Reporter Gene Constructs

Mammalian cells are generally transfected with two or three different types of plasmids. The expression plasmid contains the ER, which is constructed by ligating the cDNA of the ER gene into a eukaryotic expression vector that contains a promoter, the human growth hormone transcription termination and polyadenylation signals, the SV-40 origin of replication, and an antibiotic resistance gene for selection. A number of genes with different termination and polyadenylation signals have been used in the various expression constructs used for *in vitro* ER TA studies. The reporter plasmid contains the *Luc* gene regulated by an ERE from the frog or

fish vitellogenin gene, with a response element that is upstream of the promoter derived from a mammalian cell (e.g., the *tk* gene or the mouse mammary tumor virus long terminal repeat [MMTV]). Rather than *Luc*, a few investigators have measured ER-induced TA using the *CAT* gene that is regulated by the pS2 or *tk* promoter. A third plasmid, when used, contains the cytomegalovirus (CMV) promoter upstream of β-*gal*, which induces the transcription of β-galactosidase in the cell. The β-*gal* plasmid is used to monitor transfection efficacy and toxicity of the test substance.

Before 1996, investigators were unaware that two forms of hER existed; thus, publications before that date stated simply that hER was used. After that date, most investigators stated explicitly whether they transfected cells with hERα or hERβ. It is assumed for those publications in which it is not stated, that the hERα gene was used since this gene is the predominant form in most female reproductive organs containing hER.

2.2.2 Stably and Transiently Transfected Cell Lines

The majority of *in vitro* ER TA studies abstracted for this BRD used transiently transfected cells, despite the fact that a new batch of transfected cells must be produced for each new experiment. Transfection is performed by exposing the cells to both the ER and reporter gene plasmids in the presence of calcium phosphate, liopofectamine, or commercially available transfection reagents, or by electroporation. These substances and electroporation increase cell membrane permeability, allowing for the passive uptake of the plasmids by the cells. These foreign DNAs are typically rejected by the cell within three to seven days after transfection. In cells that harbor an endogenous or stably transfected ER, only the reporter gene construct and, if used, the construct to assess cytotoxicity, need to be transfected. Some investigators have used antibiotic selection to manipulate the cell lines so that both the expression and reporter plasmids are stable. These stably transfected cell lines do not require genetic manipulation or cell transfection skills before performing the assay.

2.2.3 *In Vitro* Mammalian Cell ER TA Assays with a Reporter Gene

The following section provides a generic example of how *in vitro* mammalian cell reporter gene ER TA assays are typically conducted.

Mammalian cells, at a confluency appropriate for the cell type, are seeded into culture dishes or wells of microtiter plates and cultured for 18-24 hours at 37°C. The cells are then transfected with the appropriate plasmids. After incubation from four to 24 hours at 37°C to express the ER, the medium is removed and the cells are treated with the test substance dissolved in culture medium, absolute ethanol, or dimethyl sulfoxide (DMSO). The cells are incubated for a further 24-48 hours at 37°C, after which time the medium is aspirated, the cells are washed with an appropriate buffer, and then lysed with the same buffer containing $MgCl_2$, Triton X 100, and dithiothreitol. After 15 minutes at room temperature, followed by centrifugation for a short time to sediment cell debris, an aliquot of the supernatant is removed for measurement of the induction of the reporter gene product.

For the induction of luciferase, adenosine triphosphate (ATP) and coenzyme A are added in glycylglycine buffer to the cell lysate in a microtiter plate. Luciferin is added to start the reaction and the fluorescence measured using a microtiter plate luminometer. The data are expressed in relative light units. For the induction of CAT, an aliquot of the lysed cells is incubated with chloramphenicol and acetyl coenzyme A, one of which is radiolabeled (Gorman et al., 1982), for 30 minutes at 37°C, and samples are removed at various time points. The reaction is stopped with ethyl acetate, which extracts the radiolabeled, acetylated chloramphenicol. The organic (ethyl acetate) phase is dried, redissolved in ethyl acetate, and spotted on silica gel plates. The radioactive acetylated product is separated from the parent chloramphenicol using thin layer chromatography. The radioactive spots are located by autoradiography of the plates for 18 hours, cut out, and counted in a scintillation counter. More recently, a commercially available ELISA kit has been used to measure CAT activity. When β-galactosidase is used as a measure of toxicity, it is measured using chlorophenol red-β-D-galactopyranoside (CRGP) as the substrate. Following hydrolysis of CRGP by β-galactosidase, the intensity of the colored product is measured at 570 nm after a 30-minute reaction period, using a spectrophotometer.

In agonism studies, the cells are treated with a test substance and the induction of the reporter gene product is used to measure the response. To assess relative potency, the maximal fold-increase induced by the test substance can be compared with that induced by the reference

estrogen. Alternatively, when dose-response data are generated, the EC_{50} for the test substance can be calculated and compared with that for the reference estrogen. The reference estrogen is included to demonstrate the adequacy of the test system and for an assessment of relative potency.

For antagonism studies, the cells are exposed simultaneously to the reference estrogen and the test substance, while control cells are exposed to the reference estrogen only. The difference in induction of the reporter gene product in the presence and absence of the test substance is used as a measure of antagonism.

2.3 Yeast Cell ER TA Reporter Gene Assays

2.3.1 Expression and Reporter Gene Constructs

For yeast ER TA assays, the expression plasmid containing the ER is under the control of the CUP1 metallothionein promoter. Copper must be added to the medium to initiate synthesis of the ER. The reporter plasmid contains palindromic ERE's inserted upstream of the promoter of the CYC1 gene linked to the *LacZ* gene that codes for β-galactosidase. Generally, hER has been used, but one publication reported on the use of the mER and three publications reported using the rtER. Four publications specifically stated that ERα and/or ERβ were transfected into the yeast cells.

2.3.2 Yeast Cell ER TA Assays with a Reporter Gene

The following section provides a generic example of how yeast cell ER TA reporter gene assays are typically conducted.

S. cerevisiae containing a stably transfected hER and a construct containing the β-*gal* reporter gene are grown overnight at 30°C in an orbital shaker in appropriate selective medium. The next day, an aliquot of the overnight culture is grown to mid-log phase, and then diluted to an OD of 0.03 at 600 nm. The diluted yeast suspension is aliquoted into a microtiter plate or small tubes and the test substance dissolved in ethanol or DMSO is added. Because hER in these cells is linked to the CUP1 promoter, 50 µM of copper sulfate ($CuSO_4$) is added to the yeast culture to induce receptor production. The cells are incubated overnight at 30°C with vigorous shaking and

the OD is read at 600 nm to assess cell growth or toxicity. A diluted aliquot of the cells is then pipetted into a microtiter plate. Assay buffer containing the color reagent, *o*-nitrophenylgalactoside (OPNG), and a lysing solution (containing sodium dodecyl sulfate [SDS], mercaptoethanol, and oxalyticase) is added to the cells. The increase in production of o-nitrophenol by the induced β-galactosidase is measured at 420 nm using a microtiter plate reader. The OD is also measured at 550 nm to correct for colorimetric distortion due to debris. β-Galactosidase activity is calculated according to the Miller equation where T = minutes of

$$\text{Miller Units } (A_{420}/\text{min/mL cells}/OD_{600}) = 1000 \times \frac{OD_{420} - (1.75 \times OD_{550})}{T \times V \times OD_{600}}$$

reaction time and V = volume of assay in mLs. Data are expressed in various formats, including absorbance at 550 nm versus the log of the molar concentration; relative potency versus the response with estrogen; β-*gal* activity (% of control versus log concentration); and fold induction of β-*gal* activity compared to induction by the reference estrogen.

In agonism studies, the yeast cells are treated with the test substances and the induction of β-galactosidase is measured. A positive response is indicated by a dose-related increase in the induction of β-galactosidase. For an assessment of relative potency, the induction may be compared to the results from a reference estrogen. For antagonism studies, the cells are exposed simultaneously to the test substance and the reference estrogen; control cells are exposed to the reference estrogen only. The difference in β-galactosidase activity in the presence and absence of the test substance is used as a measure of estrogen antagonism.

2.4 *In Vitro* Mammalian Cell ER TA Assays using Growth as an Endpoint

Although most *in vitro* ER TA studies have usually measured transcription of a reporter gene to assess ER-dependent transcriptional activation, investigators have also used assays that measure cell proliferation in a cell line in response to exposure to estrogens. In these cell lines, stimulation of cell growth is a consequence of the activation of relevant genes by estrogenic substances, presumably through a receptor-binding and activation mechanism. Although cell proliferation is not a direct measure of TA *per se*, it is a measure of the cellular consequence of the interaction of the estrogen-ligand complex with ERE. The process is relevant also to the understanding of the biochemical effects of estrogens on reproductive tissues, because one of the

outcomes of estrogen stimulation *in vivo* is tissue growth. One study considered for this BRD investigated the proliferative potential of four different MCF-7 cell lines, (BUS, ATCC, BB, BB 104), and found that the BUS cell line was the most responsive to estrogens (Villalobos et al., 1995). Besides various MCF-7 cell lines, the T47D, BG-1, ELT3, and ZR-75 cell lines have been used to measure cell proliferation. These four cell lines also express an endogenous ER (**Table 2-3**).

The following section provides a generic example of how ER TA assays with mammalian cells containing endogenous ER are typically conducted when cell proliferation is used as the endpoint.

Mammalian cells containing an endogenous hER are seeded in 12-well plates at a concentration of 20,000-50,000 cells per well, in an appropriate medium (e.g., Delbecco's modified Eagle's medium) containing 5% fetal bovine serum (FBS), and grown for 24 hours at 37°C to allow the cells to attach to the plastic surface (Soto et al., 1995). The number of cells seeded can vary according to the specific protocol and cell type used. The medium is removed and replaced with fresh medium lacking phenol red and containing FBS that has been charcoal stripped to remove contaminating hormones. Various concentrations of the test substance are added and the cells are allowed to grow for six days at 37°C. A number of procedures can be used to quantitate total cell growth. These include, for example, counting cell number, or by staining and then lysing the cells and measuring a colored dye absorbed by the cells. In one dye method, the cells are fixed and stained by treating the plates with cold trichloroacetic acid at 4°C for 30 minutes, washing the cells with tap water, and allowing them to dry. The fixed cells are stained for 10 minutes with 0.4% sulforhodamine dissolved in 1% acetic acid, washed with 1% acetic acid, and air dried. The bound dye is solubilized with 10 mM Tris base, pH 10.5, in a shaker and aliquots of each well placed in a microtiter plate and read at 492 nm (Skehan et al., 1990).

The parameter of growth generally calculated is relative proliferative effect (RPE). This parameter is calculated as the ratio (x100) between the concentration of the reference estrogen and the test substance that was required to elicit a maximal cell yield after seeding an appropriate number of cells/well. Alternatively, the relative proliferative potency (RPP) is calculated. This

is the ratio of the minimal concentration of the reference estrogen needed for maximal cell yield to the minimal concentration of test substance needed to obtain a similar effect.

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3.0 CHARACTERIZATION OF SUBSTANCES TESTED IN *IN VITRO* ER TA ASSAYS

3.1 Introduction

In vitro ER TA data were collected for a total of 698 substances that had been evaluated for their ability to activate or inhibit transcription of estrogen-inducible genes. The data were obtained from 86 peer-reviewed, scientific journal articles and two submitted reports containing unpublished data. Relevant information on the substances tested (i.e., chemical name, Chemical Abstract Service Registry Number [CASRN], chemical supplier or source, and purity) was extracted from the publications and reports and entered into a database. While the two unpublished reports included all of this information, many of the publications did not. For publications in which only chemical structures were provided, every effort was made to identify chemical names and CASRNs. The CASRNs were obtained from various sources, including the National Library of Medicine's ChemID database and *The Merck Index*. However, no attempt was made to determine the source or purity of test substances if this information was not provided. Different studies often used a unique chemical name for the same substance. When this occurred, the most commonly used chemical name was chosen and assigned to the substance, regardless of the name used in a particular publication or report, and the unique chemical nomenclature was entered into the database as a synonym (**Appendix C**).

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

The majority of the *in vitro* ER TA studies described in this BRD were conducted between 1995 and 2001. In these studies, various substances were tested for their potential to act as ER agonists or antagonists or used to study characteristics of specific ER-sensitive cell models. Most of the substances tested were industrial chemicals, pesticides, phytoestrogens, and environmental contaminants. Typically, these studies also reported on the utility of a particular *in vitro* ER TA assay as a screen for endocrine disruptor activity.

Some of the tested substances, particularly the natural estrogens (e.g., estrone, estriol) and synthetic anti-estrogens (e.g., ICI 182780, ICI 164384, hydroxytamoxifen), were studied to obtain a better understanding of their different potencies and biological activities. Some of the synthetic anti-estrogens were investigated in *in vitro* ER TA studies to evaluate their mechanisms

of action as therapeutic agents, and to determine why some of these substances have both agonist and antagonist activities.

A number of the 17β-estradiol analogs (e.g., estratriene-1,17β-diol, 1-aminoestratriene-17β-ol) were investigated to determine SAR for the development of quantitative SAR (QSAR) models. Certain pesticide and polycyclic aromatic hydrocarbon metabolites (e.g., *p,p'*-DDE, *p,p'*-DDD, 1-hydroxy-benzo[a]pyrene, 3-hydroxy-benzo[a]pyrene, 2,2-bis-(*p*-hydroxyphenyl)-1,1,1-trichloroethane) were tested to determine which metabolite enhanced or inhibited ER-induced TA and/or to provide information relating to SAR.

A few of the substances tested in *in vitro* ER TA assays were selected to address basic research questions regarding the nature of ER binding and TA processes. With the discovery of a second subtype of the ER (i.e., ERβ) in 1996, some of these substances were tested to determine whether they interacted preferentially with ERα or ERβ. These types of studies investigated the steps involved in ER activation or inhibition of target genes, and used both naturally-occurring ER-binding substances (i.e., steroids and phytoestrogens) and synthetic ER agonists and antagonists.

3.3 Chemical and Product Classes Tested

Chemical and product class information for the substances tested in *in vitro* ER TA assays is provided in **Appendix C**. Substances were assigned to chemical classes based on available information from standardized references (e.g., *The Merck Index*) and from an assessment of their chemical structures. As shown in **Table 3-1**, the chemical classes that have been tested most extensively are polychlorinated biphenyls, organochlorines, polycyclic aromatic hydrocarbons, phenolic steroids, nonphenolic steroids, phthalates, phenols, and alkylphenols. Of the 698 substances included in this BRD, 682 could be assigned to at least one chemical class, while 76 could be assigned to two chemical classes, and two could be assigned to three chemical classes.

Table 3-1 Major Chemical Classes Tested in *In Vitro* ER TA Assays*

Chemical Class	Number of Substances
Alkylphenol	30
Alkylphenyl ether	6
Aromatic hydrocarbon	6
Benzophenone	10
Biphenyl	12
Bisphenol	19
Chalcone	5
Chlorinated cyclodiene	7
Diphenylalkane	5
Flavone	10
Hydroxybenzoic acid	4
Imidazole	4
Isoflavone	19
Nitrile	4
Organochlorine	71
Organophosphate	4
Organothiophosphate	6
Paraben	6
Phenol	39
Phthalate	37
Polybrominated diphenyl ether	17
Polychlorinated biphenyl	82
Polycyclic aromatic hydrocarbon	69
Pyrethrin	6
Pyrethroid ester	5
Resorcyclic acid lactone	6
Salicylic acid	9
Steroid, nonphenolic	47
Steroid, phenolic	55
Stilbene	18
Thiophene	4
Triazine	8
Triphenylethylene	4
Urea	4

*Includes only those chemical classes for which at least four substances had been tested in *in vitro* ER TA assays.

Product classes were assigned based on information from *The Merck Index* and the National Library of Medicine's ChemFinder. A wide range of product classes is represented, as shown in **Table 3-2**. The most common product classes tested in *in vitro* ER TA assays are pesticides (including their metabolites and degradation products), pharmaceuticals, chemical intermediates, dielectric fluid components, natural products (including several phytoestrogens), and plasticizers.

Of the substances included in this BRD, 266 had no known commercial use, so were not classified within a product class.

Table 3-2 Major Product Classes Tested in *In Vitro* ER TA Assays*

Product Class	Number of Substances
Antimicrobial agent or Disinfectant	9
Antioxidant	11
Chemical intermediate	49
Cosmetics	3
Dielectric fluid	41
Dye, Pigment, or Stain	4
Flame retardant/Flame retardant additive	18
Flavor	7
Food or Food additive	5
Fragrance, Fragrance ingredient, or Perfume	8
Monomer	3
Natural product (e.g., phytoestrogens)	30
Pesticide (including metabolites and degradation products)	132
Pharmaceutical/Pharmaceutical additive	111
Plant growth regulator	3
Plasticizer	23
Preservative	15
Reagent (analytical, colorimetric, sulfhydryl)	3
Solvent	12
Surfactant	5
UV light absorber	9
Not classified	266

*Includes only those product classes for which at least three substances had been tested in *in vitro* ER TA assays.

4.0 REFERENCE DATA

In vitro ER TA assays measure the ability of a test substance to initiate or block transcription of a reporter gene, or to induce cell proliferation or inhibit its induction by a reference estrogen, in an appropriate cell line. The ability of a test substance to activate or inhibit estrogen-induced transcriptional activation *in vitro* suggests, but does not demonstrate, the ability of the substance to act *in vivo* as an estrogen agonist or antagonist.

The purpose of this BRD is to assess the performance of various *in vitro* ER TA assays with regard to their sensitivity for detecting weak ER agonists and antagonists and their reliability within and among laboratories and across procedures. No attempt is made to evaluate their performance with respect to results obtained in *in vivo* endocrine disruptor assays (e.g., the rodent uterotrophic assay). Such comparisons will be addressed elsewhere. Therefore, no reference data are included for measuring the biological relevance of the *in vitro* ER TA assays.

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5.0 DATA ON *IN VITRO* ER TA ASSAYS

5.1 Introduction

Data and methodology information were collected from 86 publications and two submitted unpublished reports on substances that had been evaluated *in vitro* in a reporter gene or cell proliferation assay for their ability to act as an ER agonist and/or antagonist. Where provided, the specific information extracted for each tested substance included its name, source, purity, methodological details, and relevant data. If available, a CASRN was identified for each substance. This identifier was obtained from various sources, including the source publication, the National Library of Medicine's ChemID database, and *The Merck Index*. Chemical name synonyms were collected for substances that were identified in the literature by more than one name, and for substances where the name used in the publication may have been different from the generic name. All substances with the same CASRN are listed under the same name, usually the common name, regardless of the name that was used in the original publication. No attempt was made to identify the source and purity of a substance if the investigators did not provide such information. **Appendix C** provides information on the names, synonyms, CASRN, and chemical/product class, if identified, for each substance. **Appendix D** contains the *in vitro* ER TA data, organized by chemical name, CASRN, and type of assay.

5.2 Availability of Detailed *In Vitro* ER TA Protocols

The Methods sections in the *in vitro* ER TA publications and the two unpublished reports provided various levels of detail. Relevant method parameters were extracted from each source and summarized in **Appendix A**. Details about the following method parameters are included in the Appendix to the extent this information was available:

- *Characteristics of cell line or yeast strain* (e.g., name of cell line/yeast strain and the source of the cell line);
- *Plasmids used to transfect cells* (e.g., ER source, ER expression vector, reporter vector, endpoint measured, plasmid transfected for cell toxicity measurements, and transfection method [i.e., whether stable or transient] and procedures);
- *Preparation of cells for assay* (e.g., growth of cells before transfection, plating time prior to treatment of cells with a test substance); and

- *Assay type* (e.g., reporter gene or cell proliferation assay, agonism and/or antagonism, solvent(s) used, test substance concentration, test substance exposure duration, reference estrogen and its concentration, number of replicates per experiment, number of times assay was repeated).

5.3 Availability of *In Vitro* ER TA Data

In vitro ER TA data for a total of 698 unique substances are included in **Appendix D**. In this Appendix, italicized values for quantitative measures of agonist or antagonist activity indicate values estimated from graphically presented data. The following *in vitro* ER TA reporter gene and cell proliferation assays were used to generate the data included in this BRD.

1. *Yeast ER TA reporter gene assays:* Data generated using 22 different *in vitro* yeast ER TA reporter gene assays involving at least 13 different strains of yeast (most likely *S. cerevisiae*) are included in this BRD. **Table 2-1** provides information, when specified in the publication or the report, on the yeast strain used, the ER source, the ER subtype, the plasmids transfected, and the corresponding designation used in this BRD. Strains stably transfected with ER (predominantly hERα) and a β-galactosidase expression vector were used in these studies.

2. *Mammalian cell ER TA reporter gene assays:* As provided in **Appendix D**, 12 different mammalian cell lines and several variants were used in *in vitro* ER TA reporter gene assays to assess the ability of a test substance to express ER agonist or antagonist activity (see **Table 2-2**). The MCF-7 cell line, derived from a human breast cancer, has been used most frequently; other human cell lines used include BG-1 (human ovarian carcinoma), HEC-1 (human endometrial tumor), HEK293 (human embryonal kidney), HeLa (human cervical tumor), HepG2 (human liver tumor), Ishikawa (human endometrial tumor), MDA-MB-231 (human breast tumor), T47D (human breast tumor), and ZR-75-1 (human breast tumor). In addition, CHO-K1 (Chinese hamster ovary), COS-1 (monkey kidney cells), and ELT-3 (rat uterine leiomyoma cells) have been used. The majority of these assays used a transiently transfected *Luc* reporter gene; however, stably transfected *Luc* or transiently/stably

transfected *CAT* genes were also used. Some laboratories transfected the cells with the β-*gal* gene as an internal control to assess transfection efficiency and cell toxicity.

3. *Mammalian cell ER TA proliferation assays:* Three cell lines and several variants that exhibit increased levels of cell proliferation in response to estrogen have been used to measure the potential estrogenicity of substances. The cell proliferation assays included in this BRD, organized by cell line, are provided in **Table 2-3**. The majority of these assays used the MCF-7 cell line.

In studies that evaluated the potential agonism of a substance in an *in vitro* ER reporter gene assay, enzyme (i.e., luciferase; CAT; β-galactosidase) activity was used as a measure of ER-induced TA. To assess agonism potency, reporter gene enzyme levels induced by the test substance were typically compared to those produced by the reference estrogen, predominantly 17β-estradiol. The quantitative results from these *in vitro* ER TA studies were most commonly presented in terms of relative activity. However, the definition of relative activity varied greatly among the reports. Relative activity was expressed as:

- Miller Units in some yeast reporter gene assay studies (see **Section 2.3.2**);
- The ratio of the response of the reference estrogen to that of the test substance (sometimes termed relative potency and calculated as [EC_{50} 17β-estradiol/EC_{50} test substance] x 100);
- Percent maximal response;
- The concentration of the test substance that produced a certain percent response relative to the reference estrogen;
- The concentration of test substance that produced a specified fold-induction (e.g., 10-fold induction of enzyme activity) over background; and
- Fold induction of enzyme activity produced by the test substance relative to the activity in the untreated controls.

These quantitative measures of agonism, if available, were extracted from the publications and reports. In reports where an EC_{50} value was not provided but dose response data were presented, the EC_{50} values of the test substance and the reference estrogen were estimated. Such data are provided in **Appendix D** in the "Agonism (Relative Activity)" and the "Agonism (EC_{50} μM)"

columns. Normalizing these values for comparison across assays was not attempted. Instead, data from each study was assigned a qualitative response of positive or negative for the particular assay system and is provided in **Appendix D** in the "Agonism (Qualitative)" column.

ER cell proliferation studies reported results as cell number, foci/cm^2, EC_{50} values, cell growth relative to hormone free control, increase in protein or DNA content, and fold increase in cell number relative to vehicle control. Data from each study was assigned a qualitative response of positive or negative for the particular assay system, as shown in **Appendix D** in the "Cell Growth" column.

The antagonism studies that used reporter gene expression or cell proliferation measured the inhibition of reference estrogen-induced enzyme activity or cell growth, respectively, by the test substance. The IC_{50} value was often presented as a measure of response. These values are summarized in **Appendix D** in the "Antagonism (Relative Activity)" column. In reports where an IC_{50} value was not provided but dose response data were presented, the IC_{50} values of the test substance and the reference estrogen were estimated. These estimated IC_{50} values are italicized in **Appendix D**. Where an IC_{50} value was not reported or a dose-response curve was not presented, test substances were assigned a qualitative response of "positive" or "negative" in the assay system used, as shown in **Appendix D** in the "Antagonism (Qualitative)" column.

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

Of the 698 substances tested in the *in vitro* ER TA assays considered in this BRD, 534 were tested in mammalian cell/yeast reporter gene agonism assays and 174 were tested in mammalian cell/yeast reporter gene antagonism assays. The substances tested in five or more mammalian cell/yeast reporter gene assays are provided in **Table 5-1**. Only 42 substances were tested for agonism in five or more mammalian cell/yeast reporter gene assays, while only eight substances were tested in five or more mammalian cell/yeast reporter gene antagonism assays. The greatest number of different mammalian cell/yeast reporter gene agonism assays used to test the same substance was eighteen, for Bisphenol A. The greatest number of different mammalian cell/yeast reporter gene antagonism assays used to test the same substance was thirteen, for ICI 182,780. More than 50% of substances tested in mammalian cell/yeast reporter gene agonism

assays (317 of 534substances; 59.4%) were tested in one assay only. Similarly, about half (76 of 174; 43.7%) of the substances tested in the reporter gene antagonism assays were tested in one assay only.

A total of 312 substances were tested in ER cell proliferation agonism assays; 67 were tested in ER cell proliferation antagonism assays. Only 47 substances were tested for ER agonism in two or more cell proliferation assays, while only eight were tested in two or more ER cell proliferation antagonism assays (**Table 5-2**). The greatest number of different cell proliferation agonism assays used to test the same substance was four, for bisphenol A, bisphenol A dimethacrylate, and estrone, while the greatest number of different cell proliferation antagonism assays used to test the same substance was three, for ICI 182,780.

A majority of the substances tested in ER cell proliferation agonism assays (265of 312 substances; 85%) were tested in one assay only. Fifty-nine of the 67 (88%) substances tested in ER cell proliferation antagonism assays were tested in one assay only.

Table 5-1 Substances Tested in Five or More *In Vitro* Mammalian Cell or Yeast ER TA Reporter Gene Assays

Substance	Number of Agonism Assays	Number of Antagonism Assays
Bisphenol A	18	
Diethylstilbestrol	17	
Methoxychlor	17	
Genistein	16	5
o,p'-DDT	14	
ICI 182,780	9	13
HPTE	12	5
4-Hydroxytamoxifen	10	12
p-Nonylphenol	11	
Zearalenone	11	
Coumestrol	10	5
2',3',4',5'-Tetrachloro-4-biphenylol	9	
2',4',6'-Trichloro-4-biphenylol	9	
Biochanin A	9	5
Daidzein	9	
Tamoxifen	9	8
Dibutyl phthalate	8	
Testosterone	8	
4-*t*-Octylphenol	7	
Bis(2-ethylhexyl)phthalate	7	
Butyl benzyl phthalate	7	
Dieldrin	7	
Estrone	7	
Kepone	7	
p,p'-DDT	7	
Equol	6	
Formononetin	6	
o,p'-DDD	6	
p,p'-DDE	6	
α,β-Endosulfan	6	
ICI 164,384	5	6
17α-Estradiol	5	
17α-Ethinyl estradiol	5	
5α-Dihydrotestosterone	5	
Atrazine	5	
Dexamethasone	5	
Ethanol	5	
Ethinyl estradiol	5	
Naringenin	5	
Nonylphenol	5	
α-Zearalanol	5	
β-Zearalenol	5	

Abbreviations: DDT = Dichlorodiphenyltrichloroethane; *o,p'*-DDD = 1,1-Dichloro-2-(*o*-chlorophenyl)ethane; *p,p'*-DDE = 1,1-Dichloro-2,2-bis[*p*-chlorophenyl]ethylene; HPTE = 2,2-Bis-(*p*-chlorophenyl)-1,1,1-trichloroethane

Table 5-2 Substances Tested in Two or More *In Vitro* ER TA Cell Proliferation Assays

Substance	Number of Agonism Assays	Number of Antagonism Assays
Bisphenol A	4	
Bisphenol A dimethacrylate	4	
Estrone	4	
Butyl benzyl phthalate	3	
ICI 182,780		3
17α-Estradiol	2	
17α-Ethinyl estradiol	2	
2-Chlorobiphenyl	2	
2,2’,5,5’-Tetrachlorobiphenyl	2	2
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	2	
2,4-Dichlorophenol	2	
2,5-Dichloro-4’-biphenylol	2	
2,5-Dichlorobiphenyl	2	
2’,4’,6’-Trichloro-4-biphenylol	2	
2-Chlorobiphenyl	2	
3,3’,5,5’-Tetrachlorobiphenyl	2	2
3,5-Dichloro-4’-biphenylol	2	
3,5-Dichlorobiphenyl	2	
4-Chlorobiphenyl	2	
Apigenin	2	
Bis(2-ethylhexyl)phthalate	2	
Butylated hydroxyanisole	2	
Butylated hydroxytoluene	2	
Chlorpyrifos	2	
Coumestrol	2	2
Dibutyl phthalate	2	
Dicofol	2	
Dieldrin	2	
Diethyl phthalate	2	
Diethylstilbestrol	2	
Dihexyl phthalate	2	
Diisobutyl phthalate	2	
Diisodecyl phthalate	2	
Diisononyl phthalate	2	
Dimethyl sulfoxide	2	
Di-*n*-butyl phthalate	2	
Ditridecyl phthalate	2	
Estriol	2	
Ethanol	2	
Flavone	2	
Genistein	2	2
Glabridin	2	2
Heptachlor	2	

Substance	Number of Agonism Assays	Number of Antagonism Assays
Octylphenol	2	
Raloxifene	2	2
Tamoxifen	2	2
Zearalenone	2	
α-Endosulfan	2	
α-Zearalanol	2	
β-Endosulfan	2	

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

Based on the available information, it appears that none of the *in vitro* ER TA studies included in this BRD used coded chemicals. Only Xenobiotic Detection Systems, Inc. stated that its studies were conducted in compliance with GLP guidelines.

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6.0 *IN VITRO* ER TA TEST METHOD PERFORMANCE ASSESSMENT

6.1 Introduction

The ICCVAM Submission Guidelines (ICCVAM, 1999) request information supporting the assessment of test method performance (i.e., accuracy, sensitivity, specificity, positive and negative predictivity, and false positive and false negative rates[1]). The ability of the new test method to predict the effect of interest is typically compared to the reference test method currently accepted by regulatory agencies. Where feasible, an assessment is made of the ability of the new method to directly predict adverse health outcomes in the species of interest (e.g., humans, wildlife). Currently, there are no methods accepted by regulatory authorities to assess ER-induced TA, and data on endocrine disruption in humans or wildlife are too limited to be used for this purpose. Thus, the approach taken to evaluating the performance of such assays is to compare the data from existing *in vitro* ER TA assays against each other with regard to their ability to detect ER agonists and antagonists.

6.2 Quantitative Assessment of Assay Performance

A quantitative analysis of the relative performance of the approximately 113 *in vitro* ER TA assays (yeast reporter gene assays = ~13 yeast strains with at least 22 assay variants; mammalian cell reporter gene assays = 12 mammalian cell lines with at least 81 assay variants; mammalian cell proliferation assays = three cell lines with at least 10 assay variants) considered in this BRD was not conducted (see ***In Vitro* ER Binding Assay BRD, Section 6**). The major reason was the almost unlimited permutations among *in vitro* ER TA assays in regard to the mammalian cell line or yeast strain used, the nature and source of the ER, the nature and type of the reporter gene (for reporter gene assays), the type of transfection (stable or transient), the experimental protocol for detecting agonistic or antagonistic activity, and the numerous and varied approaches used by the various investigators to express *in vitro* ER TA assay results. These factors, combined with

[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 (ICCVAM, 1997).

the relatively few substances tested in multiple assays, precluded a quantitative analysis. The numbers of substances tested for agonism and antagonism activity in various *in vitro* ER TA mammalian cell/yeast reporter gene assays and the mammalian cell proliferation assays are tabulated in **Table 6-1**.

6.3 Qualitative Assessment of Assay Performance

A qualitative comparative assessment of assay performance that considered the relative abilities of the various *in vitro* ER TA assays to identify substances that induced or inhibited TA was conducted. The qualitative assessment was performed separately for *in vitro* ER TA agonism and antagonism test methods. In conducting this assessment, it was assumed that there were no false positive study results. Inspection of the *in vitro* ER TA database (**Appendix D**) suggests that negative calls for some substances in some assays could be the result of limitations in protocol design (i.e., the highest dose tested might have been inadequate) rather than due to intrinsic differences in assay sensitivity. However, no effort was made to account for this possible limitation in the qualitative assessments of assay performance.

Combining the results obtained in different *in vitro* ER TA assays was not possible because of differences in the ability of the cell lines to metabolize hormones and xenobiotics, the source of the ER and type of reporter genes, as well as the possible differences in ER-induced TA levels depending on whether the ER was endogenous or transiently or stably transfected. It has been reported that the intracellular concentration of ER molecules is higher in transiently transfected cell lines than in cell lines expressing the receptor either endogenously or after it has become stabilized. The ERs transfected into the mammalian cell lines and yeast strains were derived from humans, rats, mice, or rainbow trout. In addition, ER that coded for a fusion protein of the binding domain or the ERα and ERβ from human and mouse were used by some investigators. An additional difference between the cell lines is their intracellular concentration of other hormone receptors (e.g., glucocorticoid [GR])

Table 6-1 Number of Substances Tested in Multiple *In Vitro* Mammalian Cell/Yeast ER TA Reporter Gene Assays

	Number of Assays																		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**Total**
Number of Substances Tested for Agonism	214	80	20	12	9	10	3	4	0	3	1	1	0	1	2	1	0	1	362
Percentage	59.1	22.1	5.5	3.3	2.5	2.8	0.8	1.1	0	0.8	0.3	0.3	0	0.3	0.6	0.3	0	0.3	100
Number of Substances Tested for Antagonism	89	76	9	3	4	1	1	0	0	0	1	1	0	0	0	0	0	0	185
Percentage	48.1	41.1	4.9	1.6	2.2	0.5	0.5	0	0	0	0.5	0.5	0	0	0	0	0	0	100

(**Table 6-3**), which can modulate the level of ER-induced TA. Modulation can occur in a cell if the test substance can bind to the GR and if MMTV response elements are used in the reporter vector, since it has been reported that the ligand-GR complex can interact with these elements.

Table 6-2 Number of Substances Tested in Multiple *In Vitro* ER TA Cell Proliferation Assays

	Number of Assays				
	1	**2**	**3**	**4**	**Total**
Number of Substances Tested for Agonism	265	43	1	3	312
Percentage	84.9	13.8	0.32	0.96	100
Number of Substances Tested for Antagonism	59	7	1	0	67
Percentage	88.1	10.4	1.5	0	100

Table 6-3 Characteristics of Cells Used in *In Vitro* ER TA Assays

Cell Line	Stable Transfection		Transient Transfection		Steroid Metabolizing Enzymes	Other receptors
	EXP	REP	EXP	REP		
BG-1	*	Yes	No	No		
CHO-K1†	Yes	Yes	Yes	Yes	Metabolize vinclozolin	
COS-1	No	No	Yes	Yes		
ELT-3	No	No	Yes	Yes		Progesterone
HEC-1	No	No	Yes	Yes		Progesterone
HEK293†	Yes	No	Yes	Yes		
HeLa†	Yes	Yes	Yes	Yes		
HepG2	No	No	Yes	Yes		No ERα or ERβ
Ishikawa	No	No	Yes	Yes		Progesterone
MCF-7	*	No	No	Yes		Progesterone
MDA-MB-231	No	No	Yes	Yes		
T47D	*	No	No	Yes		Progesterone
Yeast	Yes	Yes	No	No		
ZR-75	*	Yes	No	No		Progesterone

Abbreviations: EXP = Expression plasmid; REP = Reporter plasmid.
*The ER is endogenous in this cell line.
†In some cases the expression plasmid has been stably transfected and in others, it is transient.

Based on this rationale, the studies were organized by the cell line/type used. The assays were further divided into whether they harbored the hERα or hERβ gene or one of the other ERs used in various studies. The majority of reporter gene assays used luciferase synthesis to assess ER-induced TA; CAT activity was used in the other studies. The data, separated by agonism and antagonism assays, are provided in **Appendix D**.

Since very few substances were tested in multiple assays, test substance responses in mammalian cell reporter gene assays, yeast reporter gene assays, and mammalian cell proliferation assays were grouped separately and a comparison made between these three broad groupings to determine whether there were obvious differences in performance (i.e., positive, negative) based on the type of assay (reporter gene versus cell proliferation) or the target cell population (mammalian versus yeast) (**Appendix E**). For this qualitative analysis, agonism and antagonism responses were considered separately. Only substances tested in at least two of these broad groups of assays were included in the analysis. In terms of testing for ER agonism, a total of 99 substances were tested in at least one mammalian cell and at least one yeast ER TA reporter gene assay; 105 substances were tested in at least one mammalian cell ER TA reporter gene assay and at least one ER TA cell proliferation assay; and 98 substances were tested in at least one yeast ER TA reporter gene assay and in at least one ER TA cell proliferation assay. With regard to testing for ER antagonism, only 12 substances were tested in at least one mammalian cell and at least one yeast ER TA reporter gene assay; 26 substances were tested in at least one mammalian cell ER TA reporter gene assay and at least one ER TA cell proliferation assay; and 19 substances were tested in at least one yeast ER TA reporter gene assay and in at least one ER TA cell proliferation assay. In conducting this qualitative assessment, it was assumed that there were no false positive calls, even in situations where multiple tests were conducted and the number of positive calls was in the minority. This approach was used because of possible limitations in some assays associated with testing substances at relatively low concentrations only, which might have led to false negative results. The agonism assay results are presented in **Table 6-4**; the antagonism assay results in **Table 6-5**.

Table 6-4 Concordance of *In Vitro* ER TA Agonism Assays

	Agonism Assay Response*				
	Negative/ Negative	**Negative/ Positive**	**Positive/ Negative**	**Positive/ Positive**	**Total**
Mammalian Cell ER TA vs Yeast ER TA Reporter Gene Assays	11	12	9	67	99
Percentage	11.1	12.1	9.1	67.7	100
Mammalian Cell ER TA Reporter Gene vs Cell Proliferation Assays	23	8	25	49	105
Percentage	21.9	7.6	23.8	46.7	100
Yeast ER TA Reporter Gene vs Mammalian Cell Proliferation Assays	11	1	25	61	98
Percentage	11.2	1.0	25.5	62.2	100

*The first and second classifications (negative, positive) in each column refer to the first and second sets of assays, respectively, listed in each row.

When *in vitro* mammalian cell and yeast ER TA reporter gene agonism assays are compared, the results for 21 (21.2%) of the 99 substances tested in common were discordant, with about the same proportion of substances classified as negative/positive or positive/negative in both sets of assays. In contrast, 33 (31.4%) of the 105 substances tested in both mammalian cell ER TA reporter gene and cell proliferation assays were discordant, with the majority of discordant results being associated with a positive response in mammalian cell ER TA reporter gene assays and a negative response in the mammalian cell proliferation assays. A similar finding was observed for yeast ER TA reporter gene assays compared to mammalian cell proliferation assays. Based on this approach, the mammalian cell ER TA proliferation assays do not perform as well as either the mammalian cell or yeast ER TA reporter gene assays, while the two sets of reporter gene assays appear to have about equal performance.

The available data for *in vitro* ER TA antagonism assays are too limited for any conclusion about relative performance to be made.

Table 6-5 Concordance of *In Vitro* ER TA Antagonism Assays

	Antagonism Assay Response*				
	Negative/ Negative	**Negative/ Positive**	**Positive/ Negative**	**Positive/ Positive**	**Total**
Mammalian Cell vs Yeast ER TA Reporter Gene Assays	3	2	2	5	12
Percentage	25	16.7	16.7	41.7	100
Mammalian Cell ER TA Reporter Gene vs Cell Proliferation Assays	1	3	4	18	26
Percentage	3.8	11.5	15.4	69.2	100
Yeast ER TA Reporter Gene vs Mammalian Cell Proliferation Assays	7	6	0	6	19
Percentage	36.8	31.6	0	31.6	100

*The first and second classifications (negative, positive) in each column refer to the first and second sets of assays, respectively, listed in each row.

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

- The large number of assay permutations used in each category of assays;
- The lack of replicate assay test data for most of the substances considered;
- The lack of a common set of substance tested in multiple assays; and
- The assumption that positive results were more accurate than negative results.

6.4 Performance of *In Vitro* ER TA Assays

The *in vitro* ER TA assays that would be the most useful as screening tests for endocrine disrupting substances are those that are the most sensitive (i.e., have the ability to detect weak agonists and antagonists) and the most reliable within and among laboratories (see **Section 7**). In addition, it might be anticipated that 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, experimental animals for predicting the results of *in vivo* test methods) might be the most informative. Since none of these assays required the use of animals, animal welfare is not a

consideration. Finally, when taking human health and safety issues into consideration, assays that do not use radioactivity would have the greatest utility. Only a few investigators that measured CAT activity to assess ER-induced TA utilized radioactivity. However, an ELISA assay for this enzyme is now available, eliminating the need for radioactivity if this reporter gene system is used.

A qualitative evaluation of the responses of the same substances tested in the same laboratory but in different assays indicated that generally the same outcome was obtained (**Appendix F**). The major discordant results are described below.

An agonism study by Connor et al. (1997) suggested that a HeLa cell line harboring a stable hER and a stable luciferase reporter construct was more sensitive to certain hydroxylated polychlorinated biphenyls than an MCF-7 cell line in which the plasmids were transiently transfected. However, it must be noted that besides the difference in transient and stably transfected plasmids, the cell lines and reporter genes differed in these assays. Thus, it is not possible to discern which of these differences contributed to the difference in outcomes.

In an agonism study by Klotz et al (1997), three carbamate pesticides elicited a positive response in MCF-7 cells that had been transiently transfected with the Luc reporter gene but a negative response in Ishikawa cells that had been transiently transfected with the same reporter gene. This finding suggests that MCF-7 cells are more sensitive to these compounds than are Ishikawa cells.

Six xenoestrogen compounds (formononentin, tectoridin, sissotorin, 5-methoxygenistein, 7-methoxygenistein, irisolidone) induced a positive ER TA response in yeast Y190 cells transfected with the hERβ but not when the same strain was transfected with hERα (Morito et al., 2001b). In contrast to these findings, Meyers et al. (1999) reported that the hERα was more sensitive to certain chrysene derivatives than was the hERβ.

With only these few qualitative differences in responses between different assays within a laboratory, it is not possible to identify which assays are consistently more sensitive. Thus,

based on the very limited data available, there is no single reporter gene assay that can be concluded to perform better than any other assay. However, it might be anticipated that mammalian cell reporter gene assays would be preferred over similar yeast assays, simply because of differences in the increased ability of test substances to cross the mammalian cell membrane compared to the yeast cell wall (Krall and Yamamoto, 1996; Gray et al., 1997).

The mammalian cell lines used in the various *in vitro* ER TA reporter gene assays differ from each other in a number of characteristics (**Tables 2-1** and **6-2**). One important difference is whether the cell line contains expression and/or reporter genes that are stable or whether these constructs have to be transfected into the cells prior to each experiment. The COS-1, HEC-1, HeLa, and HepG2 cells used by a number of investigators were all transiently transfected with both expression and reporter plasmids prior to each experiment (**Table 2-1**). Although the MCF-7 cell line contains an endogenous ER, some investigators transfected an intact ER (Ramamoorthy et al., 1997a,b; Charles et al., 2000a,b) or the sequences coding for the binding (def) domain (Fertuck et al, 2001a,b) into this cell line. However, since the cells in which the "def" domains were transfected lacked other regions of the protein that contribute to TA, assays using only the binding domain may not be as useful as those assays using the entire protein.

6.5 Strengths and Limitations of *In Vitro* ER TA Assays

Data from *in vitro* ER TA assays indicate whether a substance can interact with the target receptor which, in turn, binds to responsive elements in DNA that initiate transcription of genes related to hormone-stimulated events in the cell. In contrast to binding assays, the TA assays provide sufficient evidence to conclude whether a substance is an ER agonist or antagonist. However, neither the binding nor the TA assay takes into consideration other cellular or organismic mechanisms that may lead to endocrine disruption (Zacharewski, 1998).

The *in vitro* ER TA reporter gene assays can be important components of a battery of screening tests because they:

- Use eukaryotic cells, many of which are derived from human tissues;
- Are cost-effective;
- Are rapid and relatively easy to perform;

- Are based on an easily quantitated, well-elucidated mechanism of action (i.e., binding to a specific protein and initiating the transcription of ER-responsive genes);
- 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 limitations of these assays include:

- The efficiency of transfection for transiently transfected cells can vary from assay to assay and laboratory to laboratory;
- The responsiveness of transiently transfected cells lasts for only a few days (Terouanne et al., 2000); and
- Inability to distinguish between the regulation of gene transcription by binding to DNA versus other mechanisms.

For yeast-based assays, additional limitations include:

- Transfected yeast lines are more prone to genetic drift over time than mammalian cells (Joyeux et al., 1997);
- Transport of test substances through the yeast cell wall might be more difficult than transport through a mammalian cell membrane, increasing the likelihood of false negative results; and
- Yeast cells may have steroid metabolic pathways that differ from mammalian cells (Gaido et al., 1997).

False positive results could occur if the cells are unable to detoxify chemicals that are usually detoxified *in vivo*, or for antagonism studies, by test substance-induced cytotoxicity that is not taken into consideration. False negative results could occur if the cell line used lacks the enzymes present *in vivo* that would normally activate the test substance to a reactive intermediate that then binds to the ER. The metabolic competency of the various cell lines (except for HepG2) is not very well characterized. The addition of the enzymes and co-factors required for metabolic activation to the assay can help to eliminate this limitation. This approach has been used in three studies in which ER-induced TA was assessed (Charles et al., 2000b; Elsby et al., 2001; Sumida et al., 2001).

Other reasons for obtaining a false negative response would be incomplete solubility of the test substance in the medium, or the presence of different co-activators in the different cell lines. Not known or addressed by any investigator is the role of co-activators in the assessment of ER-induced TA using these artificial systems. If these co-activators affect the rate of TA in these systems, the response elicited by the same substance might differ among cell lines.

6.6 Summary and Conclusions and Recommendations

Relatively few substances have been tested for ER agonism or antagonism by more than one investigator in the same *in vitro* ER TA assay, or in multiple *in vitro* ER TA assays. Consequently, much of the published data are of limited value in terms of a relative analysis of assay performance. This prevents an accurate assessment of the effectiveness and limitations of *in vitro* ER TA assays.

Based on the limited data available, there is no single *in vitro* ER TA assay that can be concluded to perform better than any other assay. However, it might be anticipated that mammalian cell assays would be preferred over yeast assays, simply because of differences in the ability of test substances to cross the mammalian cell membrane compared to the yeast cell wall. Taking various factors into consideration, it would seem that a cell line stably transfected with both hER expression and luciferase reporter plasmids (e.g., T47D) would offer the greatest utility in terms of eliminating the need to continuously prepare multiple batches of transiently transfected cells, thereby eliminating one potential source of inter-laboratory variability.

Formal validation studies should be conducted using appropriate substances, covering the range of expected responses for ER agonists and antagonists from strong to weak to negative. Testing of substances encompassing a wide range of agonist or antagonist responses are needed to adequately demonstrate the performance characteristics of any *in vitro* ER TA test method recommended as a screening assay. A list of potential test substances for use in validation efforts is provided in **Section 12**.

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7.0 *IN VITRO* ER TA TEST METHOD RELIABILITY ASSESSMENT

7.1 Introduction

The ICCVAM Submission Guidelines (ICCVAM, 1999) request information about assessment of test method reliability[1]. This information includes an evaluation of the rationale for selecting the substances used to evaluate intra- and inter-laboratory reproducibility, the extent to which the substances tested represent the range of possible test outcomes, and a quantitative statistical analysis of intra- and inter-laboratory reproducibility. In addition, information on measures of central tendency and variation for historical negative and positive control data should be included. However, no formal validation studies to assess the reliability of *in vitro* ER TA assays have been conducted and the limited nature of the current database for these assays precludes a formal analysis.

7.2 Assessment of *In Vitro* ER TA Assay Reliability

Although many of the reports indicated that the substances tested in *in vitro* ER TA assays were tested in triplicate or quadruplicate within an experiment and that at least replicate assays were conducted, associated error terms were often not provided and/or could not be estimated or calculated. Also, data analysis and presentation varied considerably among investigators assessing the *in vitro* ER agonist and antagonist activity of test substances. These two factors, combined with the great variability in assay protocols, the few substances tested multiple times within and across laboratories (and assays), and the lack of any validation studies made any formal assessment of assay reliability impractical.

For *in vitro* ER TA studies conducted to assess agonism activity, quantitative data in the form of EC_{50} values were reported for 86 and 79 substances tested in mammalian cell and yeast reporter gene assays, respectively (**Table 7-1**); 25 of these substances were tested in both mammalian cell and yeast reporter gene assays. Of these 19 substances, only six substances (biochanin A,

[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 (intra-laboratory reproducibility) or in different laboratories (inter-laboratory reproducibility) using the same protocol.

dieldrin, estriol, estrone, methoxychlor, 2',3',4',5'-tetrachloro-4-biphenylol, and 2',4',6'-trichloro-4-biphenylol) had median EC_{50} values that were higher in the mammalian cell reporter gene

Table 7-1 Median EC_{50} Values for Substances Tested for Agonism Activity in *In Vitro* ER TA Reporter Gene and Cell Proliferation Assays

Substance	Median EC_{50} Values (μM)		
	Mammalian Cell Reporter Gene Assays	Yeast Reporter Gene Assays	Mammalian Cell Proliferation Assays
2-Aminoestratriene-3,17β-diol			0.558
4-Aminoestratriene-3,17β-diol			0.346
1-Aminoestratrien-17β-ol			28.4
2-Aminoestratrien-17β-ol			0.127
3-Aminoestratrien-17β-ol			1.5
4-Aminoestratrien-17β-ol			2
4-*tert*-Amylphenol		50	
5α-Androstane-3β,17β-diol			4.66
5-Androstenediol			1.41
Benz[*a*]anthracene	4		
Benzophenone-3			3.73
Benzo[*a*]pyrene	0.815		
Biochanin A	2	0.2	
2-Biphenylol		103.5	
3-Biphenylol		40	
4-Biphenylol		5.5	
Bisdesoxyestradiol		1.64	201
Bis(4-hydroxyphenyl) ethane	1.85		
Bis(4-hydroxyphenyl) methane	1.75		
Bisphenol A	0.4	0.71	
Bisphenol B	0.088		
2,2-Bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane	0.04	0.089	
2-Bromo-4-(2,4,6-tribromo phenoxy)phenol	0.00002		
4-*sec*-Butylphenol		666	
4-*t*-Butylphenol		250	
Chlordane	6.24		
4-Chloro-3-methylphenol		378	
Chrysene	5.5		
Clomiphene		9.97	
Coumestrol	0.015	0.02	

Substance	Median EC_{50} Values (μM)		
	Mammalian Cell Reporter Gene Assays	**Yeast Reporter Gene Assays**	**Mammalian Cell Proliferation Assays**
p-Cumylphenol	0.3215		
Daidzein	0.29	17.5	0.4
Daidzin		100	
o,p'-DDD		3320	
Dihydroxy-DDE	0.074		
Monohydroxy-DDE	0.67		
o,p'-DDE		5340	
p,p'-DDE			3
o,p'-DDT	0.66	1.2	0.8
p,p'-DDT		1.01-3.27	5
4-*sec*-Decylphenol		2	
3,3'-Dibromobisphenol A	0.000025		
Dibutyl phthalate		74	
Dicofol		3	
Dieldrin	24.49	20	
cis,cis-Diethyldihydroxy tetrahydrochrysene	0.0265		
R,R-cis,cis-Diethyldihydroxy tetrahydrochrysene	0.004		
S,S-cis,cis-Diethyldihydroxy tetrahydrochrysene	0.2		
Diethylphthalate		384	
Diethylstilbestrol	0.0000189	0.000353	
Dihydrogenistein		1.025	
Dihydroglycitein		10	
5α-Dihydrotestosterone		0.431	
2,4'-Dihydroxy-4,6-dimethoxydihydrochalcone			2
4,4'-Dihydroxy-2,6-dimethoxydihydrochalcone			0.6
Diisobutylphthalate		102	
2,6-Diisopropylnaphthalene		53	
2,4-Dinonylphenol		100	
4-*sec*-Dodecylphenol		2000	
α-Endosulfan			10
α,β-Endosulfan	5.92	20	
β-Endosulfan			10
Equilin	0.0000403	0.21	
Equol	0.027	0.21	
17α-Estradiol	0.0000456	0.0046	0.0079
17β-Estradiol	0.0001 (29)	0.000175 (20)	0.00001(6)
Estradiol benzoate		0.0018	
17β-Estradiol sulfate		0.02	
6,8-Estrapentaene-3,17β-diol			0.0125

Substance	Median EC_{50} Values (μM)		
	Mammalian Cell Reporter Gene Assays	**Yeast Reporter Gene Assays**	**Mammalian Cell Proliferation Assays**
6-Estratetraene-3,17β-diol			0.00419
7-Estratetraene-3,17β-diol			0.00154
9-Estratetraene-3,17β-diol			0.099
Estratriene-3,17α-diol			0.382
Estratriene-1,17β-diol			1.59
Estratriene-2,17β-diol			0.033
Estratriene-3,11α,17β-triol			0.0365
Estratriene-3,11β,17β-triol			0.0163
Estratriene-3,16α-diol			0.237
Estratriene-3,6α,17β-triol	0.003		0.186
Estratriene-3,6β,17β-triol	0.03		0.0571
Estratriene-4,17β-diol			4.76
Estratriene-3,7α,17β-triol			0.153
Estratriene-3,7β,17β-triol			0.0122
Estratrien-17β-ol			0.316
Estratrien-3-ol		0.0032	0.118
Estriol	0.00071	0.0348	0.0483
Estrone	0.0032	0.0021	0.0772
Ethinyl estradiol	0.000011	0.002	
Fenarimol		12	2
Formononetin	0.3	16	
Genistein	0.062	0.25	
5-OMe-Genistein		20	
Genistin		10	
Glycitein		15.5	
4-*n*-Heptylphenol		13.3	
4-*tert*-Heptylphenol		0.6	
2',3,3',4',5,5'-Hexachloro-4-biphenylol	3.8		
β-Hexachlorocyclohexane			3
Hexestrol	0.0002		
4-*tert*-Hexylphenol		1	
Homosalate			1.56
3-Hydroxy-benzo[*b*] phenanthro(2,3-*d*)thiophene	0.1		
1-Hydroxybenzo[*a*]pyrene	3.2		
3-Hydroxybenzo[*a*]pyrene	0.21		
7-Hydroxybenzo[*a*]pyrene	0.43		
9-Hydroxybenzo[*a*]pyrene	0.8		
2-Hydroxychrysene	0.063		
2-Hydroxyestradiol		0.0195	
16α-Hydroxyestriol	0.001		
2-Hydroxyestriol	2		

Substance	Median EC_{50} Values (μM)		
	Mammalian Cell Reporter Gene Assays	**Yeast Reporter Gene Assays**	**Mammalian Cell Proliferation Assays**
11β-Hydroxyestrone	0.1		
2-Hydroxy-5-methylchrysene	0.32		
6-Hydroxytetralin		70	
ICI 164,384		14.5	
2-Iodoestratrien-17β-ol			1.7
3-Iodoestratrien-17β-ol			0.174
4-Iodoestratrien-17β-ol			1.67
Irisolidone		25	
11-Ketoestratriene-3,17β-diol			8.97
6-Ketoestratriene-3,17β-diol			0.00338
7-Ketoestratriene-3,17β-diol			0.016
Levonorgestrel	0.33		
Melengesterol acetate		0.11	
Methoxychlor	8.85	4.45	
3-(4-Methylbenzylidene) camphor			3.02
4-Methyl-2-nonylphenol		6	
Methyltestosterone	0.0108		
3-Monobromobisphenol A	0.00002		
Monohydroxymethoxychlor	0.198		
Nafoxidine		7.72	
Naringenin	1		
2-Nitroestratriene-3,17β-diol			33.5
4-Nitroestratriene-3,17β-diol			0.9
1-Nitroestratrien-17β-ol			60.4
2-Nitroestratrien-17β-ol			29.3
3-Nitroestratrien-17β-ol			2.16
4-Nitroestratrien-17β-ol			34.2
Nonylphenol		1.15	
n-Nonylphenol		0.8	
p-Nonylphenol	0.0845	4.8	
4-Nonylphenol diethoxylate		666	
Norethindrone	0.0281		
Norgestrel	0.242		
19-Nortestosterone	0.212		
Octylphenol		0.2	
Octyldimethyl-*p*-aminobenzoic acid			2.63
Octyl methoxycinnamate			2.37
4-*tert*-Octylphenol		0.1	
2',3,3',4,4'-Pentachloro-2-biphenylol	>4.5		

Substance	Median EC_{50} Values (μM)		
	Mammalian Cell Reporter Gene Assays	**Yeast Reporter Gene Assays**	**Mammalian Cell Proliferation Assays**
2,3,3',4',5-Pentachloro-4-biphenylol	4		
2',3',4,4',5-Pentachloro-3-biphenylol	>4.5		
2,3',4,4',5-Pentachloro-3-biphenylol	4.3		
2',3,3',4',5-Pentachloro-4-biphenylol	>4.7		
2',3,4',5,5'-Pentachloro-4-biphenylol	4.5		
3,3',4',5,5'-Pentachloro-4-biphenylol	>4.5		
4-*n*-Pentylphenol		10	
4-Phenoxyphenol	0.0000058		
1-Phenyl-3,5-*p*-hydroxy phenyl-4-ethylpyrazole	0.1		
Phloretin	0.3		
Polybrominated diphenyl ether 100	2.5		
Polybrominated diphenyl ether 119	3.9		
Polybrominated diphenyl ether 30	3.4		
Polybrominated diphenyl ether 32	5.1		
Polybrominated diphenyl ether 51	3.1		
Polybrominated diphenyl ether 71	7.3		
Polybrominated diphenyl ether 75	2.9		
4-Propyl-1,3,5-tris(4-hydroxyphenyl)pyrazole	0.0006		
4-Propylphenol		4000	
Simazine	3		
β-Sitosterol		49.2	
Tectorigenin		4.1	
Testosterone		50.9	
2,2',6,6'-Tetrachlorobiphenyl			2
2',3',4',5'-Tetrachloro-3-biphenylol	4.1		
2',3',4',5'-Tetrachloro-4-biphenylol	>4.7	0.3	0.72
3,3',5,5'-Tetrachloro-4,4'-biphenyldiol	4.3		

Substance	Median EC_{50} Values (μM)		
	Mammalian Cell Reporter Gene Assays	**Yeast Reporter Gene Assays**	**Mammalian Cell Proliferation Assays**
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	0.00003		
4-(2,4,6-Tribromophenoxy)phenol	0.0001		
2,4,6-Trichloro-4'-biphenylol	>4.7	0.08	0.22
3,3',4'-Trichloro-4-biphenylol	>4.6		
α-Zearalanol	0.00010	0.035	
β-Zearalanol		0.135	
Zearalanone		0.071	
α-Zearalenol		0.021	
β-Zearalenol	0.015	0.28	
Zearalenone	0.00343	0.096	

Abbreviations: DDE = 1,1-Dichloro-bis[4-chlorophenyl]ethylene;
DDT = Dichlorodiphenyltrichloroethane

assays than in the corresponding yeast reporter gene assays. For the cell proliferation assays, EC_{50} values were reported for 61 substances; only 13 substances were tested also in either mammalian cell or yeast reporter gene assays. The EC_{50} values for nine of these substances were higher in the mammalian cell proliferation assays than in either the mammalian cell or yeast reporter gene assays.

A comparison of the EC_{50} values of seven substances, including the reference estrogen 17β-estradiol, that were tested in all three sets of assays suggests that the mammalian cell reporter gene assays were more sensitive than the yeast reporter gene assays or the mammalian cell proliferation assays for a majority of the substances (**Table 7-2**). However, except for 17β-estradiol, EC_{50} values for most of the other substances were limited in number, with values being reported in only two to four different assays.

EC_{50} values for substances tested multiple times in the same assay in the same laboratory or in different laboratories were available only for methoxychlor, bisphenol A, 4-*tert*-octylphenol, nonylphenol, *p*-nonylphenol, genistein, *o,p'*-DDT, and 2,2-Bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane (**Table 7-3**). The reproducibility of the EC_{50} values for most of these substances within a laboratory was generally good. However, the EC_{50} values for the same substance tested

in the assay but in different laboratories could vary by two orders of magnitude. This database is extremely small but it suggests that assay reliability needs to be further evaluated.

Table 7-2 Comparison of Median EC_{50} Values (μM) for Substances Tested for Agonism Activity in *In Vitro* Mammalian and Yeast ER TA Reporter Gene and Cell Proliferation Assays

Substance	CASRN	Mammalian Cell Reporter Gene Assays	Yeast Reporter Gene Assays	Mammalian Cell Proliferation Assays
17β-Estradiol	50-28-2	0.0001 (29)	0.000175 (20)	0.00001 (6)
Estriol	50-27-1	0.00071(1)	0.0348(2)	0.0483(1)
Estrone	53-16-7	0.0063 (2)	0.0021 (3)	0.036 (1)
Daidzein	486-66-8	0.29 (2)	17.5 (2)	0.4 (1)
o,p'-DDT	789-02-6	0.66 (1)	1.2 (4)	0.8 (1)
2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	>4.7 (1)	0.3 (1)	0.72 (1)
2’,4’,6’-Trichloro-4-biphenylol	14962-28-8	>4.7(1)	0.08(1)	0.22(1)

Abbreviations: DDT = Dichlorodiphenyltrichloroethane
Numbers in parenthesis refer to the number of assays for which EC_{50} values were available

For *in vitro* ER TA studies conducted to assess antagonism activity, quantitative data in the form of IC_{50} values were reported for very few substances (**Appendix D**), thus no evaluation of the reliability of these assays was possible.

Based on the limited database available, no conclusions can be made about the relative reliability of the different *in vitro* ER TA assays considered in this BRD. However, these data do indicate the need for future validation studies to adequately evaluate this issue.

Table 7-3 EC_{50} Values of Substances Tested in the Same Assay in the Same Laboratory or the Same Assay in Different Laboratories

Assay	Substance	CASRN	EC_{50} Value (μM)	Reference
HepG2 hERα(T)+ Luc(T)+β-gal(T)	17β–Estradiol	50-28-2	0.003	Gaido et al. (2000)
HepG2 hERα(T)+ Luc(T)+β-gal(T)	17β–Estradiol	50-28-2	0.004	Gaido et al. (1999)
HepG2 hERα(T)+ Luc(T)+β-gal(T)	17β–Estradiol	50-28-2	0.0099	Gould et al. (1998)
HepG2 hERβ(T)+ Luc(T)+β-gal(T)	17β–Estradiol	50-28-2	0.007	Gaido et al. (2000)
HepG2 hERβ(T)+ Luc(T)+β-gal(T)	17β–Estradiol	50-28-2	0.008	Gaido et al. (1999)
MCF-7 hER(E)+CP(F)	17β–Estradiol	50-28-2	0.00001	Arcaro et al. (1999a)
MCF-7 hER(E)+CP(F)	17β–Estradiol	50-28-2	0.0003	Arcaro et al. (1999b)
MCF-7 hERαdef(T)+ Luc(T)+β-gal(T)	17β–Estradiol	50-28-2	0.00035	Fertuck et al. (2001a)
MCF-7 hERαdef(T)+ Luc(T)+β-gal(T)	17β–Estradiol	50-28-2	0.00035	Fertuck et al. (2001b)
MCF-7 mERβdef(T)+ Luc(T)+β–gal(T)	17β–Estradiol	50-28-2	0.00013	Fertuck et al. (2001a)
MCF-7 mERβdef(T)+ Luc(T)+β–gal(T)	17β–Estradiol	50-28-2	0.13	Fertuck et al. (2001b)
MCF-7(E3) hER(E)+CP	17β–Estradiol	50-28-2	0.000008	Vinggaard et al. (1999)
MCF-7(E3) hER(E)+CP	17β–Estradiol	50-28-2	0.00152	Wiese et al. (1997)
Yeast(*S.cer.*) hER(S)+βgal(S)	17β–Estradiol	50-28-2	0.00013	Rajapakse et al. (2001)
Yeast(*S.cer.*) hER(S)+βgal(S)	17β–Estradiol	50-28-2	0.00015	Odum et al. (1997)
Yeast(*S.cer.*) hER(S)+βgal(S)	17β–Estradiol	50-28-2	0.00003	Routledge and Sumpter (1996)
Yeast(*S.cer.*) hER(S)+βgal(S)	17β–Estradiol	50-28-2	0.0002	Routledge and Sumpter (1997)
Yeast(*S.cer.*) hER(S)+βgal(S)	17β–Estradiol	50-28-2	0.0006	Moffat et al. (2001)
Yeast(*S.cer.*) hERα(S)+βgal(S)	17β–Estradiol	50-28-2	0.00002	Vinggaard et al. (2000)
Yeast(*S.cer.*) hERα(S)+βgal(S)	17β–Estradiol	50-28-2	0.00004	Vinggaard et al. (1999)

Assay	Substance	CASRN	EC_{50} Value (μM)	Reference
Yeast(*S.cer.*) hERα(S)+βgal(S)	17β–Estradiol	50-28-2	0.000051	Elsby et al. (2001)
Yeast(*S.cer.*) hERα(S)+βgal(S)	17β–Estradiol	50-28-2	0.0002	Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+βgal(S)	17β–Estradiol	50-28-2	0.00011-0.00056	De Boever et al. (2001)
Yeast(*S.cer.*BJECZ) hER(S)+βgal(S)	17β–Estradiol	50-28-2	0.00074	Le Guevel & Pakdel (2001)
Yeast(*S.cer.*BJECZ) rtER(S)+βgal(S)	17β–Estradiol	50-28-2	0.0052	Le Guevel & Pakdel (2001)
Yeast(*S.cer.*BJ3503) hER(S)+βgal(S)	17β–Estradiol	50-28-2	0.00009	Ramamoothy et al. (1997b)
Yeast(*S.cer.*BJ3505) hER(S)+βgal(S)	17β–Estradiol	50-28-2	0.000225	Gaido et al. (1997)
Yeast(*S.cer.*Y190) hERα(S)+βgal(S)	17β–Estradiol	50-28-2	0.00002	Morito et al. (2001a)
Yeast(*S.cer.*Y190) hERα(S)+βgal(S)	17β–Estradiol	50-28-2	0.00025	Morito et al. (2001b)
Yeast(*S.cer.*Y190) hERβ(S)+βgal(S)	17β–Estradiol	50-28-2	0.00002	Morito et al. (2001a)
Yeast(*S.cer.*Y190) hERβ(S)+βgal(S)	17β–Estradiol	50-28-2	0.0002	Morito et al. (2001b)
Yeast(*S.cer.*) hERα(S)+βgal(S)	Methoxychlor	72-43-5	4.38	Elsby et al. (2001)
Yeast(*S.cer.*) hERα(S)+βgal(S)	Methoxychlor	72-43-5	4.45	Elsby et al. (2001)
Yeast(*S.cer.*) hERα(S)+βgal(S)	Methoxychlor	72-43-5	0.00066-10.3	De Boever et al. (2001)
HepG2 hERα(T)+ Luc(T)+β-gal(T)	Bisphenol A	80-05-7	0.218	Gaido et al. (2000)
HepG2 hERα(T)+ Luc(T)+β-gal(T)	Bisphenol A	80-05-7	0.64	Gould et al. (1998)
T47D hER(E)+Luc(S)	Bisphenol A	80-05-7	0.000033	Meerts et al. (2001)
T47D hER(E)+Luc(S)	Bisphenol A	80-05-7	0.77	Legler et al. (1999)
Yeast(*S.cer.*) hER(S)+βgal(S)	Bisphenol A	80-05-7	0.114	Routledge and Sumpter (1996)
Yeast(*S.cer.*) hER(S)+βgal(S)	Bisphenol A	80-05-7	3.9	Rajapakse et al. (2001)
Yeast(*S.cer.*) hERα(S)+βgal(S)	Bisphenol A	80-05-7	0.71	Elsby et al. (2001)
Yeast(*S.cer.*) hERα(S)+βgal(S)	Bisphenol A	80-05-7	0.8	Vinggaard et al. (2000)
Yeast(*S.cer.*) hERα(S)+βgal(S)	Bisphenol A	80-05-7	0.00072-0.854	De Boever et al. (2001)

Assay	Substance	CASRN	EC_{50} Value (μM)	Reference
Yeast(*S.cer.*) hER(S)+βgal(S)	Nonylphenol	84852-15-3	0.8	Odum et al. (1997)
Yeast(*S.cer.*) hER(S)+βgal(S)	Nonylphenol	84852-15-3	1.2	Odum et al. (1997)
Yeast(*S.cer.*) hER(S)+βgal(S)	*p*-Nonylphenol	104-40-5	0.02	Routledge and Sumpter (1996)
Yeast(*S.cer.*) hER(S)+βgal(S)	*p*-Nonylphenol	104-40-5	6.6	Routledge and Sumpter (1997)
Yeast(*S.cer.*) hER(S)+βgal(S)	4-*tert*-Octylphenol	140-66-9	0.15	Moffat et al. (2001)
Yeast(*S.cer.*) hER(S)+βgal(S)	4-*tert*-Octylphenol	140-66-9	0.01	Routledge and Sumpter (1996)
Yeast(*S.cer.*) hER(S)+βgal(S)	4-tert-Octylphenol	140-66-9	0.05	Routledge and Sumpter (1997)
Yeast(*S.cer.*Y190) hERα(S)+βgal(S)	Genistein	446-72-0	3	Morito et al. (2001a)
Yeast(*S.cer.*Y190) hERα(S)+βgal(S)	Genistein	446-72-0	16	Morito et al. (2001b)
Yeast(*S.cer.*Y190) hERβ(S)+βgal(S)	Genistein	446-72-0	0.1	Morito et al. (2001a)
Yeast(*S.cer.Y*190) hERβ(S)+βgal(S)	Genistein	446-72-0	0.25	Morito et al. (2001b)
Yeast(*S.cer.*) hER(S)+βgal(S)	*o,p'*-DDT	789-02-6	0.18	Routledge and Sumpter (1996)
Yeast(*S.cer.*) hER(S)+βgal(S)	*o,p'*-DDT	789-02-6	2.2	Rajapakse et al. (2001)
HepG2 hERα(T)+ Luc(T)+βgal(T)	2,2-Bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane (HPTE)	2971-36-0	0.051	Gaido et al. (1999)
HepG2 hERα(T)+ Luc(T)+βgal(T)	2,2-Bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	0.051	Gaido et al. (2000)
HepG2 hERβ(T)+ Luc(T)+βgal(T)	2,2-Bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	0.01	Gaido et al. (1999)
HepG2 hERβ(T)+ Luc(T)+βgal(T)	2,2-Bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	0.03	Gaido et al. (1999)
Yeast(*S.cer.*) hER(S)+βgal(S)	2,2-Bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	0.05	Odum et al. (1997)
Yeast(*S.cer.*) hERα(S)+ βgal(S)	2,2-Bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	0.128	Elsby et al. (2001)

7.3 Summary, Conclusions and Recommendations

The *in vitro* ER TA 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 agonists and antagonists, and the most reliable (i.e., exhibit the least variability within and across laboratories). Based on the available data, no valid assessment of assay reliability was possible.

Taking into account the available *in vitro* ER TA assay database, and the inability to adequately assess the reliability of the large number of *in vitro* ER TA assays considered in this BRD, formal validation studies should be conducted using appropriate substances covering a range of expected EC_{50} values (for agonism) and IC_{50} values (for antagonism). These substances should elicit a range of responses ranging from strong to weak to inactive to demonstrate the reliability characteristics of the *in vitro* ER TA assays considered as possible screening assays. A list of potential test substances for use in such validation efforts 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 the reporting and archiving of laboratory data and records, and information about the test protocol, to ensure the integrity, reliability, and accountability of a study (U.S. EPA, 1994a,b; FDA, 1994). Based on the information provided in the reports included in this BRD, the only set of *in vitro* ER TA studies conducted in compliance with GLP guidelines were conducted by Xenobiotic Detection Systems, Inc.

8.2 Assessment of Data Quality

Formal assessments of data quality, such as quality assurance audits, generally involve a systematic and critical comparison of the data provided in a study report or published paper to the laboratory records generated during the study. No attempt to formally assess the quality of the data was performed for this BRD. The published and submitted data on the TA of ER-inducible genes were limited, in most reports, to the response of the substance in the test system relative to 17β-estradiol or to a vehicle control, and to a lesser extent, EC_{50} values, and rates of enzyme activity. A number of studies used cell proliferation as a surrogate endpoint for TA; some of these studies used 17β-estradiol or another potent estrogen as a reference estrogen. Auditing these reported data and values would require obtaining the original data for each study, which are not readily available.

An informal assessment of the *in vitro* ER TA publications and the two submitted reports revealed limitations that complicate interpretation of the ER TA data (**Appendix D**):

- *Various formats used to report study results*: The data from the studies were reported in a variety of formats. Yeast-based reporter gene studies reported test results in Miller Units (A_{420}/min/mL cells/OD_{600}), potency ratios (EC_{50} test substance/EC_{50} 17β-estradiol), β-galactosidase activity, percent maximal response, and relative potency (EC_{50} 17β-estradiol/ EC_{50} test substance x 100). Studies using reporter genes in mammalian cell lines reported results as fold induction or increase, relative potency ratios, relative agonistic activity, EC_{50}

values, concentration-response curves, and rates of enzyme activity. Cell proliferation studies reported results as cell number, foci/cm^2, EC_{50} values, cell growth relative to hormone free control, increase in protein or DNA content, and fold increase in cell proliferation relative to vehicle control. The values reported were, as a rule, obtained from different protocols, and against different standards, and there typically was little or no information regarding the concentrations of ER or reporter gene constructs. These factors make a quantitative analysis of assay reliability difficult.

- *Large number of substances tested in only one laboratory*: Relatively few of the substances included in this BRD have been tested by more than one laboratory using the same protocol. Therefore, the inter-laboratory reproducibility of the results for many of the substances cannot be determined.
- *Large number of substances without information regarding within-laboratory reproducibility:* There is often no information in the published scientific articles as to the number of replicates or repeat experiments performed. Therefore, the within-laboratory repeatability of many of the test results cannot be determined.
- *Insufficient methodology information*: Many of the published studies contained limited details about the specific test protocols, cells, and vectors used. In some cases, methods were reported as being "performed as previously described," and in many of these cases the cited publication either referenced another publication for experimental details, or was not relevant to the particular protocol. Thus, for some studies, it was not possible to determine the actual protocol used to produce data.
- *Inconsistent nomenclature of test substances:* Most studies did not provide CASRNs for the substances tested, or used a unique chemical nomenclature, which in some cases made unequivocal identification of the test substance difficult.

8.3 Quality Control Audit

A quality control (QC) audit of the *in vitro* ER TA database provided in **Appendix D** was conducted. The data in the database was checked against the original sources and entry errors were corrected.

9.0 OTHER SCIENTIFIC REPORTS AND REVIEWS

9.1 Availability of Other *In Vitro* ER TA Data

Some of the peer-reviewed publications identified during the initial literature search for *in vitro* ER TA studies were not abstracted for inclusion in this BRD. The reasons for not abstracting these publications include:

- The studies lacked appropriate qualitative and/or quantitative test data;
- The test substances were not adequately identified, or were undefined mixtures; or,
- The publications contained insufficient information about the test method used.

NICEATM published a formal request in the *Federal Register* (Vol. 66, No. 57, pp.16278 – 16279) for unpublished *in vitro* ER TA data and/or information from completed studies using or evaluating *in vitro* ER TA assays. Two submissions were received in response to this request, one from Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan, and the other from Xenobiotic Detection Systems, Inc., Durham, North Carolina, U.S. The data from these unpublished submissions are included in **Appendix D,** which also contains the *in vitro* ER TA data from the published articles considered in this BRD.

A number of companies involved in pharmaceutical discovery and development routinely use *in vitro* ER TA assays to screen substances for their potential estrogenic activity. However, these data are not in the public domain and were not provided to NICEATM.

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

9.2 Conclusions from Other Scientific Reviews of *In Vitro* ER TA Methods

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

9.2.1 1996 Endocrine Disruptor Screening Methods Workshop

In vitro ER TA and cell proliferation assays were discussed 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 co-sponsored by the U.S. EPA, the Chemical Manufacturers Association (CMA), and the World Wildlife Fund (WWF).

The proceedings briefly discussed *in vitro* ER TA assays that use MCF-7 cells, a cell line derived from human mammary carcinoma cells. In these assays, ER is expressed endogenously, while a reporter gene that is linked to an ER-inducible response element is transiently transfected into the MCF-7 cells. This type of *in vitro* ER TA assay is routinely used to measure ER-induced TA. In the proceedings, it was noted that luciferase activity appears to be a more sensitive indicator of TA than CAT expression.

A variation of the MCF-7 assay described in the proceedings uses a chimeric receptor in which the hER ligand binding domain is fused to Gal4. MCF-7 cells are transiently co-transfected with a vector containing the cDNA for the chimeric receptor and a vector containing a Gal4-controlled luciferase construct. This assay was originally developed to differentiate substances that elicit estrogen activity through phosphorylation of the ER from those that activate the ER through binding. While this assay is not widely used, a variety of chemical classes, including organochlorines, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, phthalates, and alkylphenols, have been tested by the few laboratories that employ it. Gray et al. (1997) indicated that "these assays … have relatively high sensitivity."

An *in vitro* ER TA assay using stably transfected MCF-7 cells was also discussed at the workshop. The discussion focused on one stably transfected MCF-7 cell line, MVLN, which endogenously expresses hER and contains transfected genes that encode luciferase, linked to a vitellogenin promoter. As described in the proceedings, the major advantages of this assay over others that measure ER-induced TA include:

- The assay is easier to conduct than ones using transiently transfected cell lines;
- This assay has been standardized in several laboratories; and
- The assay can be used for high-throughput screening.

In addition, Gray et al. (1997) discussed the major advantages and disadvantages of yeast-based *in vitro* ER TA assays. These assays use recombinant, stably transformed yeast (*S. cerevisiae*) cells that contain the complete ER gene, or specific portions of ER from humans or other species of interest, and a reporter gene, typically *lacZ* (which encodes β-galactosidase), linked to an ER-inducible response element.

The major advantages of yeast assays, as described in the proceedings, include:

- The assay is relatively easy to perform because it uses stably transfected cells;
- The incubation time of the test substance is short, ranging from 4 hours to overnight;
- The assay has been automated for high-throughput screening; and
- The assay has been adapted to test chemical mixtures.

In the proceedings, it was noted that the workshop participants did not reach a consensus with respect to the applicability of yeast-based *in vitro* ER TA assays as an endocrine disruptor screening method. The major disadvantages of yeast-based *in vitro* ER TA assays identified by the authors include:

- They do not appear to distinguish between agonists and antagonists (e.g., the known ER antagonist, ICI 164,384, is reported to induce TA);
- There may be significant metabolic differences between yeast and mammalian cells that could make it difficult to extrapolate data from these assays to humans;
- The cell wall and chemical transport systems of yeast cells are reported to selectively maintain low intracellular concentrations of some steroid hormones, a phenomenon that may also apply to other types of substances;
- The permeability of the yeast cell wall may differ significantly from that of mammalian cell membranes; and
- Different yeast strains appear to differ in their response to estrogenic substances, the amount of ER expressed, and the uptake of test substances into the cell.

The usefulness of MCF-7 cell proliferation assays, which use cell growth to identify ER agonists and antagonists, was discussed also at the workshop. Although the assay appeared to be very

sensitive, a number of factors could affect the utility of the assay for the large-scale screening of substances. One major concern noted in the proceedings is the potential difficulty of standardizing MCF-7 cell culture procedures, which must change over time to accommodate changes in MCF-7 cells over time. Another concern is that frozen MCF-cells generally require an adaptation period (sometimes up to three months) after thawing before exhibiting their full response to estrogens. Also, the duration of the assay, about six days, is considered somewhat long for an *in vitro* assay. One other concern is the potential for false positive and false negative results from substances that promote cell growth through other mechanisms, or are cytotoxic.

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 co-sponsored a workshop in Kansas City, Missouri, U.S. that addressed the use of "gene expression" assays as a type of *in vitro* screening method for detecting potential (anti-)estrogenic substances in wildlife. Ankley et al. (1998) edited the proceedings of this workshop.

The major advantages described by the authors for using gene expression assays as endocrine disruptor screens for wildlife include:

- Assays that use eukaryotic cell lines can distinguish between agonists and antagonists;
- The assays are amenable to automation using microtiter plates, which would allow for the rapid processing of large numbers of samples; and
- The methods are amenable to standardization.

The major disadvantages include:

- The assays require specialized equipment and training;
- Transient transfection of plasmids can be labor-intensive and may contribute to decreased reliability;
- The poor correlation for some substances between yeast-based and mammalian cell-based assays; and

- These assays currently have limited applicability to non-mammalian species, which have been poorly studied with regard to development of suitable reporter gene assays for detection of (anti-)estrogenic substances.

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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 methods that refine, reduce, or replace animal use where scientifically feasible. 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 non-animal 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 TA 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 TA Assays

All of the *in vitro* ER reporter gene and cell proliferation assays addressed in this BRD utilize cultured whole cells containing estrogen-inducible gene expression systems and, therefore, do not require use of animals. From an animal welfare perspective, all of these *in vitro* cell-based assays are equally advantageous. However, because none of these assays have been validated for the routine testing of substances, further development and validation is required.

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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 inter-laboratory reproducibility.

The ICCVAM Submission Guidelines (ICCVAM, 1999) request that an assessment of test method transferability be conducted with respect to the following factors that influence transferability:

- Availability of the facilities and the fixed major 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 Fixed Major Equipment

The facilities needed to conduct *in vitro* ER TA assays are widely available, and the necessary laboratory equipment is readily available from suppliers. To ensure personnel and community safety, facilities should adhere to pertinent State or Federal regulations for the handling of hazardous substances and wastes.

The specific needs as related to the various *in vitro* ER TA procedures utilizing reporter genes, whether transiently or stably transfected, are essentially the same. These are described briefly below.

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

Fixed major equipment: Luminometer for assays requiring luciferase detection; cell incubator with temperature, CO_2, and humidity controls; sterile biohazard/safety hoods; and freezer.

Cell proliferation assays have the same facility and equipment requirements as reporter gene assays, except that cell counting equipment would be an additional requirement.

11.2 Training Considerations

Assays using stably transfected/transduced cell lines: Currently, most of the mammalian and yeast cell lines containing a stably transfected ER and a reporter are not available commercially. A high level of technical expertise would be required to establish such cell lines. However, once established in a laboratory, the cell lines could be readily used in a reporter gene assay that requires staff with basic laboratory skills and training in cell culture techniques.

Assays using transiently transfected cell lines: These assays require staff with basic laboratory skills and training in cell culture techniques and transient transfections.

Cell proliferation assays: Performing these assays requires staff with basic laboratory skills and training in cell culture and cell counting techniques.

11.3 Estimated Cost and Time Considerations

Table 11-1 provides information on the expected time needed to perform a study, special equipment needed, and other considerations. Cost information was not available for all of the assays.

Table 11-1 Comparison of Estimated Costs, Time, and Special Equipment Needs for Different *In Vitro* ER Reporter Gene and Cell Proliferation Assays

Assay	Cost/Test substance	Duration	Special Equipment	Other Considerations
BG1Luc4E2 Reporter Gene Assay -- Stable	$250 - $600	3 days	Luminescence counter (luminometer) for luciferase detection (~$20,000)	Commercially available
HeLa Reporter Gene Assay –Transient or Stable	n.a.	3-4 days	Luminescence counter (luminometer) for luciferase detection (~$20,000)	
HepG2 Reporter Gene Assay – Transient	$1950	3-4 days	Luminescence counter (luminometer) for luciferase detection (~$20,000)	
MCF-7 Focus Assay	n.a.	14 days	Cell colony counter (automated)	
MCF-7 E-SCREEN Assay	n.a.	5-7 days		Estrogenic response of different MCF-7 cell subclones varies; the most sensitive subclones may not be commercially available.
MCF-7 Reporter Gene Assay -- Transient	n.a.	3-4 days	Luminescence counter (luminometer) for luciferase detection (~$20,000)	
Yeast Reporter Gene Assay –Stable	$1600	2-3 days		

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

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12.0 MINIMUM PROCEDURAL STANDARDS FOR *IN VITRO* ER TA ASSAYS AND RECOMMENDED SUBSTANCES FOR USE IN VALIDATION STUDIES

12.1 Introduction

A relatively large number of *in vitro* studies have been published on the ability of substances to act as an ER agonist or antagonist. However, the number of substances tested multiple times in the same assays or in multiple assays is extremely limited, precluding a formal analysis of assay performance. In addition, there are no published guidelines for conducting such studies and no formal validation studies have been conducted to assess the performance or reliability of *in vitro* ER TA assays. To assist in the development and characterization of these assays, minimum procedural standards and a list of recommended test substances for use in validation studies are provided. The minimal procedural standards and recommended test substances are based on an evaluation of the multiple *in vitro* ER TA assays considered in this BRD (**Appendix D**, **Sections 6** and **7**). Based on the limited database available, it is difficult to recommend one specific assay for future validation efforts. However, for a more reliable test, it is recommended that an assay with an endogenous or stable ER, using a stable or transiently transfected reporter vector containing the *Luc* gene be used. All test substances must be tested for both agonism and for antagonism (in the presence of the reference estrogen)

12.2 Minimum Procedural Standards

The minimum procedural standards listed below are recommended for standardized protocols developed for various types of *in vitro* ER TA assays. Adequate procedural details are essential to maximize inter-laboratory reproducibility and minimize variation that may contribute to erroneous or non-reproducible results.

12.2.1 Transcriptional Activation of the Reference Estrogen

Irrespective of the source of the cell line used, the TA-inducing ability of the reference estrogen (i.e., 17β-estradiol) (**Section 12.2.2**) must be demonstrated. Consistency in the level of the reporter gene product response induced by the reference estrogen is used as a measure of the intra-laboratory reproducibility of the assay, and as a criterion for assay acceptance.

12.2.2 Reference Estrogen

Except for one study in which cell proliferation was tested, all the reports to assess ER-induced transcriptional activation used 17β-estradiol as the reference estrogen. Thus, it is recommended that investigators continue to use 17β-estradiol as the reference estrogen in future testing.

12.2.3 Preparation of Test Substances

Test substances must be dissolved in culture medium or in a solvent that is miscible with the medium. For substances not sufficiently water soluble, absolute ethanol or DMSO are proposed as solvents. Preference is given to absolute 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, or otherwise interfere, with the test system. A solvent control must be included in each 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 physico-chemical mechanisms, most testing schemes include a limit dose (i.e., the highest dose that should be tested in the absence of solubility or toxicity constraints). An agreed upon limit dose for *in vitro* ER TA screening assays has not been established. Historically, the highest dose tested in such assays has ranged from 1 to 100 μM, with most tests conducted using a maximum dose of 1 μM. The EC_{50} values reported for substances tested in various *in vitro* ER TA assays cover eight to nine orders of magnitude (from 20 pM to 8 mM), although the majority of EC_{50} values ranged from 20 pM to 100 nM. Thus, if the *in vitro* ER TA test is required to detect substances with an EC_{50} that is at least 6 orders of magnitude higher than that of 17β-estradiol, then the limit dose (unless precluded by chemical properties such as solubility) should be 100 μM. However, if five orders of magnitude are sufficient for detecting ER agonists, then the limit dose should be 10 μM.

For the *in vitro* screening for ER agonists, it is recommended that the limit dose be 100 μM and that a concentration range from 10 pM to 100 μM, in ten-fold increments, be used in each experiment. However, if it is suspected that the test substance binds weakly to the ER, the dose range should extend up to 10 mM, in ten-fold increments.

For ER antagonism assays, the weakest ER antagonist had a reported IC_{50} value of 0.1 mM. Therefore, the range of substance concentrations tested in such studies should be from 1 nM to 1 mM.

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

12.2.5 Solvent and Positive Controls

Concurrent negative and solvent controls and a reference estrogen must 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. The reference estrogen in *in vitro* ER TA agonism assays is included to demonstrate the sensitivity of the assay in each experiment for detecting agonist activity and to allow for an assessment of variability in the conduct of the assay across time. In addition, to demonstrate the sensitivity of the *in vitro* ER TA antagonism assay, a substance with demonstrated ER antagonism activity (i.e., a positive control) is needed in each experiment. ICI 182,780 is suggested as the candidate ER antagonist as this substance has historically been shown to be negative as an agonist but positive as an antagonist. For the background antagonist control, ICI 182,780 should be tested in the absence of the reference estrogen. The reference estrogen should be tested alone (positive control) and the reference estrogen with ICI 182,780 as the antagonist control.

12.2.6 Within Test Replicates

Triplicate values should be obtained for each dose tested, for each control and test substance.

12.2.7 Dose Spacing

Generally, to obtain a response curve to assess ER-induced transcriptional activation, 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 pM to 100 µM). For antagonists, the concentration range should range from 10 nM to 1 mM. This results in each test of the testing of nine concentrations for agonism and six concentrations of each substance for antagonism. If the range of doses is reduced due to, for example, insolubility of the test substance at the limit dose, then equivalent spacing (e.g., half-log doses) of the nine or six doses over the smaller dose range should be used.

12.2.8 Data Analysis

Different investigators have used various approaches for analyzing data obtained from *in vitro* ER TA assays. For agonist assays, responses are compared to the concurrent vehicle control while for antagonist assays, treatments are compared to the response induced by the reference estrogen alone. Data analysis approaches have varied from a visual inspection of the data only to more formal statistical approaches using either one- or two-way analysis of variance (ANOVA) (with main effects being treatment or replicates and treatment, respectively) using a general linearized model. In some studies, the induced reporter gene response for each replicate has been converted to a fold induction above the concurrent control level, and means and variances of these data used as the basis for analysis. EC_{50} or IC_{50} values have been calculated using various curve fitting programs. One curve fitting approach was based on a logistic dose response model where the asymptotic minimum and maximum response, the dose that is halfway between the minimum and maximum, and the slope of the line tangent to the logistic curve at this mid-point is determined (see Gaido et al., 1997). Asymptotic standard errors of the parameter estimates are employed to perform two-sided “t” tests.

It would be useful, during any future validation study, that various approaches for analyzing *in vitro* ER TA agonist and antagonist data be evaluated and compared in order to develop a standard approach.

12.2.9 Assay Acceptance Criteria

An *in vitro* ER TA assay testing for agonism activity should be accepted only if the response for the reference estrogen occurs within the appropriate confidence limits based on historical data. An *in vitro* ER TA assay testing for antagonism activity should be accepted only if the response for the reference estrogen and the positive antagonism control occur within the appropriate confidence limits based on historical data.

12.2.10 Evaluation and Interpretation of Results

A substance is classified as an ER agonist if the assay-specific response (e.g., luciferase activity) is significantly increased above the concurrent control level, as determined by an appropriate statistical test. A substance is classified as an ER antagonist if the substance induces a significant decrease in the ability of the reference estrogen to induce transcriptional activation, as determined by an appropriate statistical 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 (every attempt should be made to obtain the purity); and
- Physicochemical properties relevant to the study (e.g., solubility, stability, volatility).

Solvent:

- Justification for choice of solvent if other than medium, absolute ethanol, or DMSO;
- Information to demonstrate that the solvent, if other than medium, absolute ethanol, or DMSO, does not affect the sensitivity of the assay.

Estrogen receptor:

- Type and source of ER (if from a commercial source, the supplier must be identified);
- Isolation procedure or method for making constructs; and
- Nomenclature and components of the expression and reporter constructs.

Reporter plasmid:

- Type of reporter gene;
- Type and structure of response elements;
- Original plasmid used to make construct; and
- Description and methodology used to make plasmid that is transfected.

Cell line:

- Source of cell line and protocol for maintenance of the cell line;
- Growth parameters of the cell line before initiation of the assay; and
- Method used to transfect the reporter construct if it is transiently transfected into the cells.

Test conditions:

- Rationale for the concentration of the reference estrogen used;
- Composition of media and buffers 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 controls;
- Method used to lyse cells after incubation;
- Method used to measure transcriptional activation;
- Methods used to determine fold induction, EC_{50} value for agonism studies, or IC_{50} value for antagonism studies; and
- Statistical methods used.

Results:

- Extent of precipitation of test substance;
- Reporter response for each replicate at each dose for all test substances, including confidence levels or other measure of intra-dose repeatability;

- Calculated EC_{50} value for agonism studies or IC_{50} value for antagonism studies, and confidence limits, for the reference estrogen (agonism studies), positive control (antagonism studies), and test substance; and
- Fold increase above control for each concentration.

Discussion of the results:

- Historical fold increases in activity and EC_{50} values for reference estrogen (agonism), including ranges, means, and standard deviations
- Reproducibility of IC_{50} value of positive control antagonist compared to historical data

Conclusion:

- Classification of test substance with regard to *in vitro* ER TA agonist or antagonist 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 fold increase is marginal, the EC_{50} or IC_{50} value is not well defined, the call is equivocal, the test shows excess variability), repeat tests to clarify the results of the primary test would be prudent.

12.3 Standardization of *In Vitro* ER TA Binding Assays for Validation

Appendix B provides six *in vitro* ER TA assay protocols submitted by five investigators. The assay protocols, as titled by the investigators, are:

- Protocol for HepG2 Cells + Receptor + Reporter and/or β-gal Plasmids for Use in Steroid Hormone Receptor Assays, as provided by Dr. Kevin Gaido, CIIT Centers for Health Research, Research Triangle Park, NC, USA.
- Protocol for Chimeric ERα-Mediated Reporter Gene Expression in MCF-7 Cells, as provided by Dr. Timothy Zacharewski, Dept. of Biochemistry, Michigan State University, Lansing, MI, USA.
- Protocol for the Development of New Reporter Gene Assay Systems for Screening Endocrine Disrupters, as provided by Drs. Mitsuru Iida and Teruhisa Kato, Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan.

- Protocol for the Development of Stably Transfected Cell Lines to Screen Endocrine Disrupters, as provided by Drs. Mitsuru Iida and Teruhisa Kato, Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan.
- Technical Perspective on the U.S. EPA Endocrine Disruptor Screening Program: In Vitro EDSTAC Guideline Protocols, as provided by Dr. Grantley Charles, Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, MI, and Dr. William Kelce, Pharmacia Corporation, Kalamazoo, MI, USA.
- Lyticase-based cell lysis protocol of β-Galactosidase assay for 96 well plates, as provided by Dr. Rémy Le Guével of the Université de Rennes, Rennes, France.

Inspection of these protocols provides a perspective on how various *in vitro* ER TA assays are conducted by different investigators. These protocols provide a basis for developing a more general protocol, one that takes into account the recommended minimum procedural standards provided in **Section 12.2**. Prior to developing that protocol, the protocols in **Appendix B** need to be reviewed for completeness and adequacy for their intended purpose.

12.4 List of Recommended Substances for Validation of *In Vitro* ER TA Assays

Tables 12-1 and **12-2** provide recommended lists of substances to be used in the assessment of the reliability and comparative performance of *in vitro* ER TA agonist and antagonist assays, respectively. A number of factors were considered in developing the list for ER agonist studies, including the number of times the substance had been tested in any assay; the median EC_{50} value, when available, of the substance in all assays in which it was tested; the fold increase in response above the control substance; and whether it had been recommended for testing in the ER binding BRD. The latter was considered since it would be informative to assess whether a substance was positive for ER binding but did not elicit a positive transcriptional activation response or vice-versa. For antagonists, the median IC_{50}, if available, and the fold decrease in transcriptional activation compared to the reference estrogen was used. Selection of the substances was based on the availability and concordance of multiple test results among the multiple *in vitro* ER TA assays considered in this BRD (**Appendix E**). When quantitative data was not available for a few substances, consideration was given to qualitative responses (i.e.,

positive, a weak positive, or negative). Very few substances were tested for their antagonistic properties in these assays.

In a validation study, it is important to include substances that cover the range of possible responses and, therefore this list includes substances in each category. The variability in the numbers of strong, weak, and negative substances in each list reflects the available database.

Table 12-1 List of Substances Recommended for Validation of *In Vitro* ER TA Assays for Agonism

Substance	CASRN	Number of Mammalian Cell Reporter Gene Assays in Which Tested	Median EC_{50} Value (μM) in Mammalian Cell Reporter Gene Assays	Median RBA[a]
17α-Ethinyl estradiol	57-63-6	2	0.000011	
Diethylstilbestrol	56-53-1	8	0.0000189	200
17α-Estradiol	57-91-0	2	0.000046	
17β-Estradiol	50-28-2	46	0.0001	
α-Zearalanol	26538-44-3	2	0.00011	
Estrone	53-16-7	3	0.0032	48
Zearalenone	17924-92-4	8	0.002	44
Methyltestosterone	58-18-4	2	0.0108	
β-Zearalenol	71030-11-0	2	0.015	
Coumestrol	479-13-0	7	0.015	1.9
Estriol	50-27-1	1	0.00071	14.4
4-*tert*-Octylphenol	140-66-9	3	Not available – 0.10 in yeast	0.20
Genistein	446-72-0	11	0.062	0.56
p-Nonylphenol	104-40-5	4	0.0845	
19-Nortestosterone	434-22-0	1	0.212	
Equol	531-95-3	2	0.27	
Daidzein	486-66-8	5	0.29	
Phloretin	60-82-2	4	0.3	
Levonorgestrel	797-63-7	2	0.33	
Bisphenol A	80-05-7	13	0.399	0.056
o,p'-DDT	789-02-6	7	0.66	0.013
Naringenin	480-41-1	5	1.0	0.008

Substance	CASRN	Number of Mammalian Cell Reporter Gene Assays in Which Tested	Median EC_{50} Value (μM) in Mammalian Cell Reporter Gene Assays	Median RBA[a]
p,p'-DDT	50-29-3	3	Not available – 2.14 in yeast	0.0003
Chlordane	57-74-9	1	6.24	
Methoxychlor	72-43-5	12	8.85	0.001
Progesterone	57-83-0	2	negative	0.0003
Atrazine	1912-24-9	3	negative	0.0003
Dicofol	115-32-2	1	negative	
Fluoranthene	206-44-0	0	Not available -- negative in yeast	
Heptachlor	76-44-8	1	negative	
Mirex	2385-85-5	2	negative	

[a] RBA = Median relative binding affinity reported only for substances recommended for use in validating ER binding assays (*Current Status of Test Methods for Detecting Endocrine Disruptors: In Vitro ER Binding*); the median RBA value reported is for positive rat uterine cytosol tests.
Abbreviations: DDT = Dichlorodiphenyltrichloroethane.

Table 12-2 List of Substances Recommended for Validation of *In Vitro* ER TA Assays for Antagonism

Substance	CASRN	Qualitative Response in Mammalian Cell Reporter Gene Assays*
4-Hydroxytamoxifen	68047-06-3	positive (7)
Tamoxifen	10540-29-1	positive (6/7)**
ICI 164,384	98007-99-9	positive (4)
ICI 182,780	129453-61-8	positive (10/11)**
Raloxifene	84449-90-1	positive (5)
Kaempferide	491-54-3	positive (2)
Flavone	525-82-6	positive (2)
Droloxifene	82413-20-5	positive (4)
Hydroxytoremifine	110503-62-3	positive (4)
Dibenz[*a,h*]anthracene	53-70-3	Not available -- positive (2) in yeast
4-Octylphenol	1806-26-4	Not available – positive (1) in cell proliferation
Bendiocarb	22781-23-3	positive (1)

Substance	CASRN	Qualitative Response in Mammalian Cell Reporter Gene Assays*
Zearalenone	17924-92-4	positive (2/3)**
Apigenin	520-36-5	positive (2/4)**
Phloretin	60-82-2	positive (1/3)**
Coumestrol	479-13-0	positive (1/3)**
Formononetin	485-72-3	negative (2)
Bisphenol A	80-05-7	negative (2)
Atrazine	1912-24-9	negative (2)
Fluoranthene	206-44-0	Not available – negative (2) in yeast

Abbreviations: DDT = Dichlorodiphenyltrichloroethane
*Numbers in parentheses refer to the number of different mammalian cell reporter gene assays in which the substance was tested.
** Number of assays in which the substance was positive compared to the number of assays in which it was tested.
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12.5 Summary and Conclusions

Currently, there are no published guidelines for conducting *in vitro* ER TA studies, and no formal validation studies have been conducted to assess the reliability or performance of the currently available assays. To support the further development and characterization of *in vitro* ER TA agonism and antagonism 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 an evaluation of the *in vitro* ER TA assays considered in this BRD. It is recommended that a mammalian cell assay with an endogenous gene and stably transfected reporter gene, as well as stably transfected plasmid containing luciferase to monitor toxicity be evaluated.

The minimum procedural standards include methods for determining the ability of the reference estrogen to induce transcriptional activation; methods for establishing a stable cell line; the concentration range of the test substance (including the limit dose) to test for agonists and antagonists; the use of negative, solvent, and positive controls; the number of replicates to use; 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 are

described. These minimum procedural standards are provided to ensure that *in vitro* ER TA studies will be conducted in such a manner as to allow the results to be understandable and comparable among procedures.

Six submitted *in vitro* ER TA assay protocols developed by experts in the field are provided in **Appendix B**. Inspection of these protocols provides a perspective on how various *in vitro* ER TA assays are conducted by different investigators, and for developing a more general protocol, one that takes into account the recommended minimum procedural standards. Prior to developing that protocol, these protocols need to be evaluated for completeness and adequacy for their intended purpose.

A number of factors were considered in developing a list of substances to be used in validation efforts, including the EC_{50} and IC_{50} value of the substance in all of the assays in which it has been tested. The selected substances were sorted according to whether they were positive, weak positive, or negative in at least one *in vitro* ER TA assay.

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

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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**.

BG-1: A cell line derived from a human ovarian carcinoma.

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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.

COS: A monkey kidney cell line.

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

CV-1: A monkey kidney cell line.

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.

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

ELT-3: A rat uterine leiomyoma cell line.

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 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.

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.

HEC-1: A cell line derived from a human endometrial carcinoma.

HEK293: A cell line derived from a human embryonal kidney.

HeLa: A cell line derived from a human cervical cancer.

HepG2: A cell line derived from a human hepatoma (liver tumor).

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.

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.

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.

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

Intra-laboratory 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 performed in test tubes or petri dishes using single-cell organisms or under cell-free conditions.

In vivo: In the living organism. Refers to assays performed in multi-cellular organisms.

Ishikawa: A cell line derived from a human endometrial tumor.

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

Ligand-binding domain: The area within a receptor molecule that is designed to attract and hold a ligand.

MCF-7: Cell lines from a human mammary adenocarcinoma.

MDA (all variations)**:** A cell line derived from a human breast carcinoma.

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

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.

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

Precocious puberty: A clinical situation where boys or girls begin showing signs of puberty prior to its expected onset.

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 an 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 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 ability.

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).

Screen/Screening Test[2]**:** A 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., be able to reliable identify active chemicals but not inactive chemicals).

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

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

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

T47D: A cell line derived from a human breast tumor.

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

Tier 2 assay: An assay that is the component of the EDSP testing battery.

Transcriptional activation (assay): An assay to measure 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.

Transfection: The process by which foreign DNA is introduced into a cell to change the cell's genotype.

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.

ZR-75-1: A cell line derived from a human breast tumor.

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Appendix A
Methods for *In Vitro* ER TA Assays

A1 ER TA Reporter Gene Assays Using MCF-7 Cells

A2 ER TA Reporter Gene Assays Using Various Mammalian Cells

A3 ER TA Cell Proliferation Assays

A4 ER TA Reporter Gene Assays Using Yeast Cells

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Appendix A1

ER TA Reporter Gene Assays Using MCF-7 Cells

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ER TA Reporter Gene Assays Using MCF-7 Cells

	Bonefeld-Jørgensen et al. (2001)	**Charles et al. (2000a)**
Characteristics of Cell Line		
Cell subtype	n.p.	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	n.p.	Lipofectinâ
ER expression vector	endogenous	GAL4-HEG0 (chimeric hERαdef)
ER source	human	human
ER transfection	n.a.	Transient
Reporter vector	pERE-tk-CAT	17 m5-G-Luc
Reporter/endpoint	CAT	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pON249 (β-gal)	pCH110 (β-gal)
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.p.	6 hours
Time from transient transfection to treatment of cells	n.p.	16-18 hours
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	yes
Metabolic activation source	n.a.	S9 from Aroclor-induced male Sprague Dawley rat
Test substance solvent	Ethanol	n.p.
Range of test substance concentrations	3 to 9 µM or 0.1 nM	1 pM to 10 µM
No. replicates	3	n.p.
No. times assay repeated	3 to 4	n.p.
Agonism assay		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	10 nM	1 pM - 10 nM
Incubation time of test substance	48 hours	24 hours
Antagonism assay		not done
Reference ligand	17β-Estradiol	
Final conc., reference ligand	10 nM	
Incubation time of test substance		

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using MCF-7 Cells

	Charles et al. (2000b)	**Clemons et al. (1998)**
Characteristics of Cell Line		
Cell subtype	n.p.	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	Lipofectinâ	Calcium phosphate
ER expression vector	GAL4-HEG0 (chimeric hERαdef)	GAL4-HEG0 (chimeric hERαdef)
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	17 m5-G-Luc	17m5-G-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pCH110 (β-gal)	pCMV (β-gal)
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	6 hours	6 hours
Time from transient transfection to treatment of cells	16-18 hours	Overnight
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO
Range of test substance concentrations	10 nM to 10 µM	10 pM to 0.1 µM
No. replicates	3	2
No. times assay repeated	3 to 5	2
Agonism assay		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	1 pM - 10 nM	n.p.
Incubation time of test substance	24 hours	24 hours
Antagonism assay	not done	
Reference ligand		17β-Estradiol
Final conc., reference ligand		n.p.
Incubation time of test substance		

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using MCF-7 Cells

	Collins-Burow et al. (2000)	**Connor et al. (1996)**
Characteristics of Cell Line		
Cell subtype	M variant p250	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	Lipofect AMINE™	Calcium phosphate
ER expression vector	endogenous	GAL4-HEG0 (chimeric hERαdef)
ER source	human	human
ER transfection	n.a.	Transient
Reporter vector	pERE2-luciferase (vitellogenin ERE linked to Luc)	17m5-G-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pCMV (β-gal)	none
Other plasmid transfection	Transient	n.a.
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	Overnight	n.p.
Time from transient transfection to treatment of cells	n.p.	24 hours
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	n.p.	DMSO
Range of test substance concentrations	1, 10, 25 µM	1 nM to 10 µM
No. replicates	3	n.p.
No. times assay repeated	3	At least 3
Agonism assay		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	1 nM	0.1 pM to 10 nM
Incubation time of test substance	18 - 24 hours	24 hours
Antagonism assay		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	1 nM	1 nM
Incubation time of test substance	18 - 24 hours	24 hours

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using MCF-7 Cells

	Connor et al. (1997)	**Fertuck et al. (2001a)**
Characteristics of Cell Line		
Cell subtype	n.p.	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	n.p.	Calcium phosphate
ER expression vector	hER (undefined)	GAL4-HEG0 (chimeric hERαdef)
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	Vit-CAT plasmid	17m5-G-Luc
Reporter/endpoint	CAT	luciferase
Reporter transfection	Transient	Transient
Other plasmids	none	pCMV-lacZ
Other plasmid transfection	n.a.	Transient
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.p.	6 hours
Time from transient transfection to treatment of cells	12 hours	18 hours
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO
Range of test substance concentrations	10 µM	0.1 nM to 10 µM
No. replicates	n.p.	n.p.
No. times assay repeated	At least 3	At least 3
Agonism assay		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	1 nM	10 nM
Incubation time of test substance	48 hours	24 hours
Antagonism assay		not done
Reference ligand	17β-Estradiol	
Final conc., reference ligand	1 nM	
Incubation time of test substance		

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using MCF-7 Cells

	Fertuck et al. (2001a)	**Fertuck et al. (2001b)**
Characteristics of Cell Line		
Cell subtype	n.p.	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	Calcium phosphate	Calcium phosphate
ER expression vector	GAL4-mERβdef	GAL4-HEG0 (chimeric hERαdef)
ER source	mouse	human
ER transfection	Transient	Transient
Reporter vector	17m5-G-Luc	17m5-G-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pCMV-lacZ	pCMV-lacZ
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	6 hours	n.p.
Time from transient transfection to treatment of cells	18 hours	18 hours
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO
Range of test substance concentrations	0.1 nM to 10 µM	0.1 to 10 µM
No. replicates	n.p.	n.p.
No. times assay repeated	At least 3	At least 3
Agonism assay		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	10 nM	1 nM
Incubation time of test substance	24 hours	24 hours
Antagonism assay	not done	
Reference ligand		17β-Estradiol
Final conc., reference ligand		0.1 and 1 nM
Incubation time of test substance		24 hours

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using MCF-7 Cells

	Fertuck et al. (2001b)	**Fielden et al. (1997)**
Characteristics of Cell Line		
Cell subtype	n.p.	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	Calcium phosphate	Calcium phosphate
ER expression vector	GAL4-mERβdef	GAL4-HEG0 (chimeric hERαdef)
ER source	mouse	human
ER transfection	Transient	Transient
Reporter vector	17m5-G-Luc	17m5-G-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pCMV-lacZ	pCMV-lacZ
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.p.	6 hours
Time from transient transfection to treatment of cells	18 hours	16 hours
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO
Range of test substance concentrations	0.1 to 10 µM	0.1 to 10 µM
No. replicates	n.p.	2
No. times assay repeated	At least 3	3
Agonism assay		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	1 nM	5 nM
Incubation time of test substance	24 hours	24 hours
Antagonism assay		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	0.1 and 1 nM	1 nM
Incubation time of test substance	24 hours	24 hours

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using MCF-7 Cells

	Jobling et al. (1995)	**Klotz et al. (1996)**
Characteristics of Cell Line		
Cell subtype	n.p.	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	Calcium phosphate	Lipofect AMINE™
ER expression vector	endogenous	endogenous
ER source	human	human
ER transfection	n.a.	n.a.
Reporter vector	pERE-tk-Luc (vitellogenin A2)	pERE2-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pJ7LacZ	pCMV (β-gal)
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	to 80% confluence	72 hours
Time from transient transfection to treatment of cells	n.p.	5 hours
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	n.p.	DMSO or ethanol
Range of test substance concentrations	0.1 to 10 µM	1 pM to 20 µM
No. replicates	2	3
No. times assay repeated	2	At least 2
Agonism assay		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	10 nM	0.1 nM
Incubation time of test substance	24 hours	18 hours
Antagonism assay		not done
Reference ligand	17β-Estradiol	
Final conc., reference ligand	10 pM	
Incubation time of test substance	24 hours	

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using MCF-7 Cells

	Klotz et al. (1997)	**Kramer et al. (1997)**
Characteristics of Cell Line		
Cell subtype	n.p.	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	Lipofect AMINE™	n.p.
ER expression vector	endogenous	endogenous
ER source	human	human
ER transfection	n.a.	n.a.
Reporter vector	pERE2-Luc	pVit-tk-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Stable
Other plasmids	pCMV (β-gal)	none
Other plasmid transfection	Transient	n.a.
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	72 hours	n.a.
Time from transient transfection to treatment of cells	5 hours	n.a.
Stable transfection		
Plating time prior to treatment with test substance	n.a.	48 hours
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO or ethanol	Ethanol
Range of test substance concentrations	100 nM	0.1 to 10 µM
No. replicates	3	4
No. times assay repeated	2	n.p.
Agonism assay		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	0.5 nM	0.1 nM
Incubation time of test substance	18 hours	48 hours
Antagonism assay		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	0.5 nM	0.1 nM
Incubation time of test substance	18 hours	48 hours

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using MCF-7 Cells

	Lascombe et al. (2000)	**Matthews et al. (2001)**
Characteristics of Cell Line		
Cell subtype	MELN41	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	n.p.	Calcium phosphate
ER expression vector	endogenous	Gal4-hERαdef
ER source	human	human
ER transfection	n.a.	Transient
Reporter vector	ERE-Luc	17m-5-G-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Stable	Transient
Other plasmids	none	pCMV-lacZ
Other plasmid transfection	n.a.	Transient
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.a.	7 hours
Time from transient transfection to treatment of cells	n.a.	16 hours
Stable transfection		
Plating time prior to treatment with test substance	24 hours	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	Ethanol	n.p.
Range of test substance concentrations	100 nM to 100 µM	1 pM to 10 µM
No. replicates	2	n.p.
No. times assay repeated	3	2 to 3
Agonism assay		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	10 nM	1 pM - 10 nM
Incubation time of test substance	12 hours	24 hours
Antagonism assay		not done
Reference ligand	17β-Estradiol	
Final conc., reference ligand	10 nM	
Incubation time of test substance	12 hours	

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using MCF-7 Cells

	Matthews et al. (2001)	**Ramamoorthy et al. (1997a)**
Characteristics of Cell Line		
Cell subtype	n.p.	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	Calcium phosphate	n.p.
ER expression vector	Gal4-hERβdef	hER (undefined)
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	17m-5-G-Luc	CKB-CAT (rat creatine kinase B construct) or CATH-CAT (human cathepsin)
Reporter/endpoint	luciferase	CAT
Reporter transfection	Transient	Transient
Other plasmids	pCMV-lacZ	none
Other plasmid transfection	Transient	n.a.
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	7 hours	n.p.
Time from transient transfection to treatment of cells	16 hours	14 - 16 hours
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	n.p.	n.p.
Range of test substance concentrations	1 pM to 10 µM	10 nM to 10 µM
No. replicates	n.p.	n.p.
No. times assay repeated	2 to 3	3
Agonism assay		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	1 pM - 10 nM	10 nM
Incubation time of test substance	24 hours	14-16 hours
Antagonism assay	not done	not done
Reference ligand		
Final conc., reference ligand		
Incubation time of test substance		

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using MCF-7 Cells

	Ramamoorthy et al. (1997b)	**Sumida et al. (2001)**
Characteristics of Cell Line		
Cell subtype	n.p.	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	"transfection cocktail"	Lipofectinâ
ER expression vector	hER (undefined)	endogenous
ER source	human	human
ER transfection	Transient	n.a.
Reporter vector	CKB-CAT or CATH-CAT	pGV-tk-vEREx5
Reporter/endpoint	CAT	luciferase
Reporter transfection	Transient	Transient
Other plasmids	none	none
Other plasmid transfection	n.a.	n.a.
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.p.	n.p.
Time from transient transfection to treatment of cells	14 - 16 hours	Overnight
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	yes
Metabolic activation source	n.a.	S9 from induced rat liver
Test substance solvent	n.p.	DMSO
Range of test substance concentrations	10 nM to 10 µM	n.p.
No. replicates	n.p.	4
No. times assay repeated	3	n.p.
Agonism assay		
Reference ligand	17β-Estradiol	none
Final conc., reference ligand	10 nM	n.p.
Incubation time of test substance	48 hours	24-28 hours
Antagonism assay	not done	not done
Reference ligand		
Final conc., reference ligand		
Incubation time of test substance		

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using MCF-7 Cells

	Yoshihara et al. (2001)	**Zacharewski et al. (1998)**
Characteristics of Cell Line		
Cell subtype	n.p.	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	Transfast™	Calcium phosphate
ER expression vector	endogenous	GAL4-HEG0 (chimeric hERαdef)
ER source	human	human
ER transfection	n.a.	Transient
Reporter vector	p(ERE)3-SV40-Luc	17m5-G-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pRL/CMV	pCMV-lacZ
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.p.	n.p.
Time from transient transfection to treatment of cells	n.p.	24 hours
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Metabolic activation	yes	no
Metabolic activation source	S9 from male Wistar rat	n.a.
Test substance solvent	Ethanol	DMSO
Range of test substance concentrations	50 nM and 500 nM	100 nM to 10 µM
No. replicates	n.p.	4
No. times assay repeated	4	n.p.
Agonism assay		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	0.1 nM	10 nM
Incubation time of test substance	24 hours	24 hours
Antagonism assay	not done	not done
Reference ligand		
Final conc., reference ligand		
Incubation time of test substance		

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

Appendix A2

ER TA Reporter Gene Assays Using Various Mammalian Cells

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ER TA Reporter Gene Assays Using Various Mammalian Cells

	Balaguer et al. (1996)	**Bonefeld-Jørgensen et al. (2001)**
Characteristics of Cell Line		
Cell line	HeLa	MDA-MB-231
Cell subtype		
Cell source	human cervical cancer	human breast cancer (ER-neg)
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate precipitation	n.p.
ER expression vector	Gal4-HEGO (hERαdef)	pSG5-HEO (ERα)
ER source	human	human
ER transfection	Stable	Transient
Reporter vector	p17m5-G-Luc	pERE-LUC
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Stable	Transient
Other plasmids	pAG60 (neo)	none
Other plasmid transfection	Stable	n.a.
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.a.	n.p.
Time from transient transfection to treatment of cells	n.a.	n.p.
Stable transfection		
Plating time prior to treatment with test substance	16 hours	n.a.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	Ethanol
No. replicates	2	At least 9
No. times assay repeated	At least 3	3 or 4
Range of test substance concentrations	100 nM to 10 µM	0.1 µM or 9 µM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	100 nM to 10 µM	10 nM
Incubation time of test substance	24 hours	n.p.
Antagonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	1 nM	10 nM
Incubation time of test substance	24 hours	n.p.

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Connor et al. (1997)	**Gaido et al. (1999)**
Characteristics of Cell Line		
Cell line	HeLa	HeLa
Cell subtype		
Cell source	human cervical cancer	human cervical cancer
Transfection of Cells with Plasmids		
Transfection method/reagent	n.p.	Superfectâ
ER expression vector	Gal4-HEGO (hERαdef)	hERα (undefined)
ER source	human	human
ER transfection	Stable	Transient
Reporter vector	17m5-G-Luc	vtERE-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Stable	Transient
Other plasmids	none	pCMV-β-gal
Other plasmid transfection	n.a.	Transient
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.a.	n.p.
Time from transient transfection to treatment of cells	n.a.	n.p.
Stable transfection		
Plating time prior to treatment with test substance	16 hours	n.a.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO
No. replicates	4	3
No. times assay repeated	At least 3	3 to 4
Range of test substance concentrations	100 nM to 10 µM	1 pM to 1 µM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	1 nM	1 pM to 100 nM
Incubation time of test substance	24 hours	24 hours
Antagonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	1 nM	n.p.
Incubation time of test substance	24 hours	24 hours

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Gaido et al. (1999)	**Gaido et al. (1999)**
Characteristics of Cell Line		
Cell line	HeLa	HepG2
Cell subtype		
Cell source	human cervical cancer	human liver tumor
Transfection of Cells with Plasmids		
Transfection method/reagent	Superfectâ	Superfectâ
ER expression vector	hERβ (undefined)	hERα (undefined)
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	vtERE-Luc	C3-Luc or MMTV-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pCMV-β-gal	pCMV-β-gal
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.p.	Overnight
Time from transient transfection to treatment of cells	n.p.	n.p.
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO
No. replicates	3	3
No. times assay repeated	3 to 4	3 or 4
Range of test substance concentrations	1 pM to 1 µM	0.1 nM to 10 µM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	1 pM to 100 nM	10 pM to 100 nM
Incubation time of test substance	24 hours	24 hours
Antagonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	n.p.	n.p.
Incubation time of test substance	24 hours	24 hours

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Gaido et al. (1999)	**Gaido et al. (1999)**
Characteristics of Cell Line		
Cell line	HepG2	HepG2
Cell subtype		
Cell source	human liver tumor	human liver tumor
Transfection of Cells with Plasmids		
Transfection method/reagent	Superfectâ	Superfectâ
ER expression vector	hERβ (undefined)	rERα (undefined)
ER source	human	rat
ER transfection	Transient	Transient
Reporter vector	C3-Luc or MMTV-Luc	C3-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pCMV-β-gal	pCMV-β-gal
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	Overnight	Overnight
Time from transient transfection to treatment of cells	n.p.	n.p.
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO
No. replicates	3	3
No. times assay repeated	3 or 4	3 or 4
Range of test substance concentrations	0.1 nM to 10 µM	0.1 nM to 10 µM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	10 pM to 100 nM	10 pM to 100 nM
Incubation time of test substance	24 hours	24 hours
Antagonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	n.p.	n.p.
Incubation time of test substance	24 hours	24 hours

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Gaido et al. (1999)	**Gaido et al. (2000)**
Characteristics of Cell Line		
Cell line	HepG2	HepG2
Cell subtype		
Cell source	human liver tumor	human liver tumor
Transfection of Cells with Plasmids		
Transfection method/reagent	Superfectâ	Superfectâ
ER expression vector	rERβ (undefined)	hERα (undefined)
ER source	rat	human
ER transfection	Transient	Transient
Reporter vector	C3-Luc	C3-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pCMV-β-gal	pCMV-β-gal
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	Overnight	Overnight
Time from transient transfection to treatment of cells	n.p.	n.p.
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO
No. replicates	3	n.p.
No. times assay repeated	3 or 4	3
Range of test substance concentrations	0.1 nM to 10 µM	10 nM to 10 µM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	10 pM to 100 nM	0.6 µM
Incubation time of test substance	24 hours	24 hours
Antagonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	n.p.	0.6 µM
Incubation time of test substance	24 hours	n.p.

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Gaido et al. (2000)	**Garner et al. (1999)**
Characteristics of Cell Line		
Cell line	HepG2	HeLa
Cell subtype		
Cell source	human liver tumor	human cervical cancer
Transfection of Cells with Plasmids		
Transfection method/reagent	Superfectâ	Electroporation
ER expression vector	hERβ (undefined)	pRSV
ER source	human	mouse
ER transfection	Transient	Transient
Reporter vector	C3-Luc	ERET81CAT
Reporter/endpoint	luciferase	CAT
Reporter transfection	Transient	Transient
Other plasmids	pCMV-β-gal	none
Other plasmid transfection	Transient	n.a.
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	Overnight	n.p.
Time from transient transfection to treatment of cells	n.p.	n.p.
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO or Ethanol
No. replicates	n.p.	n.p.
No. times assay repeated	3	3
Range of test substance concentrations	10 nM to 10 µM	1 nM to 10 µM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	0.6 µM	10 nM
Incubation time of test substance	24 hours	28 hours
Antagonism		not done
Reference ligand	17β-Estradiol	
Concentration of reference ligand	0.6 µM	
Incubation time of test substance	n.p.	

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Gould et al. (1998)	Hodges et al. (2000)
Characteristics of Cell Line		
Cell line	HepG2	ELT-3
Cell subtype		
Cell source	human liver tumor	rat uterine leiomyoma
Transfection of Cells with Plasmids		
Transfection method/reagent	n.p.	Calcium phosphate
ER expression vector	hERα (undefined)	pRSVT7
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	pC3-Luc	ERE-tk-LUC6a
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pRSV-β-gal	pCMV-β-gal
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	18 hours	n.p.
Time from transient transfection to treatment of cells	n.p.	24 hours
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO or methanol	DMSO
No. replicates	3	3
No. times assay repeated	4 to 6	n.p.
Range of test substance concentrations	10 pM to 10 µM	100 nM to 100 µM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand		10 nM
Incubation time of test substance	24 hours	48 hours
Antagonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand		n.p.
Incubation time of test substance	24 hours	n.p.

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Hoogenboom et al. (2001)	**Klotz et al. (1997)**
Characteristics of Cell Line		
Cell line	T47D	Ishikawa
Cell subtype		
Cell source	human breast adenocarcinoma	human endometrial cancer
Transfection of Cells with Plasmids		
Transfection method/reagent	n.p.	Lipofect AMINE™
ER expression vector	endogenous	pSG5-hER
ER source	human	human
ER transfection	n.a.	Transient
Reporter vector	p-EREtata-Luc	pERE2luc
Reporter/endpoint	luciferase	luciferase induction
Reporter transfection	Stable	Transient
Other plasmids	none	pCMV-β-gal
Other plasmid transfection	n.a.	Transient
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.a.	72 hours
Time from transient transfection to treatment of cells	n.a.	5 hours
Stable transfection		
Plating time prior to treatment with test substance	n.p.	n.a.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO or ethanol
No. replicates	3	3
No. times assay repeated	n.p.	2
Range of test substance concentrations	.0001 to 10,000 nM	100 nM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	varied	1 nM
Incubation time of test substance	24 hours	18 hours
Antagonism	not done	
Reference ligand		17β-Estradiol
Concentration of reference ligand		1 nM
Incubation time of test substance		18 hours

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Kraichely et al. (2000)	**Kraichely et al. (2000)**
Characteristics of Cell Line		
Cell line	HEC-1	HEC-1
Cell subtype		
Cell source	human endometrial cancer	human endometrial cancer
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	Calcium phosphate
ER expression vector	pBD-GAL4 (ERα)	pBD-GAL4 (ERβ)
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	(ERE)3-pS2-CAT	(ERE)3-pS2-CAT
Reporter/endpoint	CAT expression	CAT expression
Reporter transfection	Transient	Transient
Other plasmids	pCMV-β-gal	pCMV-β-gal
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.p.	n.p.
Time from transient transfection to treatment of cells	n.p.	n.p.
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	n.p.	n.p.
No. replicates	n.p.	n.p.
No. times assay repeated	At least 3	At least 3
Range of test substance concentrations	1 pM to 1 µM	1 pM to 1 µM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	n.p.	n.p.
Incubation time of test substance	n.p.	n.p.
Antagonism	not done	
Reference ligand		17β-Estradiol
Concentration of reference ligand		n.p.
Incubation time of test substance		n.p.

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Kuiper et al. (1998)	**Kuiper et al. (1998)**
Characteristics of Cell Line		
Cell line	HEK 293	HEK 293
Cell subtype		
Cell source	human embryonal kidney	human embryonal kidney
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	Calcium phosphate
ER expression vector	pSG5-HEGO (hERαdef)	pSG5-hERβ
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	3xERE-ATAT-LUC	3xERE-ATAT-LUC
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	β-gal (not described)	β-gal (not described)
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	24 hours	24 hours
Time from transient transfection to treatment of cells	16 hours	16 hours
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	ethanol	ethanol
No. replicates	3	3
No. times assay repeated	2	2
Range of test substance concentrations	1 nM to 1 µM	1 nM to 1 µM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	n.p.	n.p.
Incubation time of test substance	24 hours	24 hours
Antagonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	n.p.	n.p.
Incubation time of test substance	18 hours	18 hours

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Legler et al. (1999)	**Makela et al. (1994)**
Characteristics of Cell Line		
Cell line	T47D	HeLa
Cell subtype		
Cell source	human breast adenocarcinoma	human cervical cancer
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	Electroporation
ER expression vector	endogenous	pRSV-ER
ER source	human	mouse
ER transfection	n.a.	Transient
Reporter vector	p-EREtata-Luc	ERET81CAT
Reporter/endpoint	luciferase	CAT expression
Reporter transfection	Stable	Transient
Other plasmids	pGK-Hyg	none
Other plasmid transfection	Stable	n.a.
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.a.	n.p.
Time from transient transfection to treatment of cells	n.a.	4 hours
Stable transfection		
Plating time prior to treatment with test substance	48 hours	n.a.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO or ethanol	n.p.
No. replicates	3	3
No. times assay repeated	n.p.	n.p.
Range of test substance concentrations	.01 to 1000 nM	100 nM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	30 pM	1 nM
Incubation time of test substance	24 hours	28 hours
Antagonism		not done
Reference ligand	17β-Estradiol	
Concentration of reference ligand	6 pM	
Incubation time of test substance	n.p.	

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Meerts et al. (2001)	**Meerts et al. (2001)**
Characteristics of Cell Line		
Cell line	T47D	HEK 293
Cell subtype		
Cell source	human breast adenocarcinoma	human embryonal kidney
Transfection of Cells with Plasmids		
Transfection method/reagent	n.p.	n.p.
ER expression vector	endogenous	pSG5-hERα
ER source	human	human
ER transfection	n.a.	Stable
Reporter vector	pEREtata-Luc	3xERE-ATAT-LUC
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Stable	Stable
Other plasmids	none	none
Other plasmid transfection	n.a.	n.a.
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.a.	n.a.
Time from transient transfection to treatment of cells	n.a.	n.a.
Stable transfection		
Plating time prior to treatment with test substance	48 hours	48 hours
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO or ethanol	DMSO or ethanol
No. replicates	n.p.	n.p.
No. times assay repeated	n.p.	n.p.
Range of test substance concentrations	10 to 10,000 nM	100 to 10,000 nM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	n.p.	n.p.
Incubation time of test substance	24 hours	24 hours
Antagonism		not done
Reference ligand	17β-Estradiol	
Concentration of reference ligand	10 pM	
Incubation time of test substance	24 hours	

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Meyers et al. (1999)	**Meyers et al. (1999)**
Characteristics of Cell Line		
Cell line	HEC-1	HEC-1
Cell subtype		
Cell source	human endometrial cancer	human endometrial cancer
Transfection of Cells with Plasmids		
Transfection method/reagent	n.p.	n.p.
ER expression vector	hERα (plasmid unspecified)	hERβ (plasmid unspecified)
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	$(ERE)_3$-pS2-CAT	$(ERE)_3$-pS2-CAT
Reporter/endpoint	CAT	CAT
Reporter transfection	Transient	Transient
Other plasmids	pCMV-β-gal	pCMV-β-gal
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.p.	n.p.
Time from transient transfection to treatment of cells	n.p.	n.p.
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	n.p.	n.p.
No. replicates	n.p.	n.p.
No. times assay repeated	At least 3	At least 3
Range of test substance concentrations	1 nM to 1 µM	1 nM to 1 µM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	10 nM	10 nM
Incubation time of test substance	n.p.	n.p.
Antagonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	1 nM	1 nM
Incubation time of test substance	n.p.	n.p.

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Miksicek (1993)	**Miksicek (1994)**
Characteristics of Cell Line		
Cell line	HeLa	HeLa
Cell subtype		
Cell source	human cervical cancer	human cervical cancer
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	Calcium phosphate
ER expression vector	pER-18	pER-18
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	pERE-TK-CAT	pERE-TK-CAT
Reporter/endpoint	CAT	CAT
Reporter transfection	Transient	Transient
Other plasmids	none	none
Other plasmid transfection	n.a.	n.a.
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	24 hours	24 hours
Time from transient transfection to treatment of cells	5-6 hours	5-6 hours
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	Ethanol	Ethanol
No. replicates	n.p.	n.p.
No. times assay repeated	n.p.	n.p.
Range of test substance concentrations	varies; 10 pM-10 nM & 1 nM-10 µM & 10 nM-10 µM	varies for each substance; from 1 pM to 10 µM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	5 nM	1 nM
Incubation time of test substance	48 hours	48 hours
Antagonism	not done	
Reference ligand		17β-Estradiol
Concentration of reference ligand		1 nM
Incubation time of test substance		48 hours

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Moore et al. (1997)	**Otsuka Pharmaceutical (2001)**
Characteristics of Cell Line		
Cell line	HeLa	CHO
Cell subtype		K1
Cell source	human cervical cancer	Chinese hamster ovary
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	n.p.
ER expression vector	Gal4-HEGO (hERαdef)	pcDNA ERα
ER source	human	n.p.
ER transfection	Stable	Transient
Reporter vector	p17m5-G-Luc	pGL3ERE-7
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Stable	Transient
Other plasmids	pAG60 (neo)	pcDNA-EGFP
Other plasmid transfection	Stable	Transient
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.a.	24
Time from transient transfection to treatment of cells	n.a.	3
Stable transfection		
Plating time prior to treatment with test substance	16 hours	n.a.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO
No. replicates	2	4
No. times assay repeated	4	2
Range of test substance concentrations	10 nM to 10 µM	varies; about 10 pM to 10 µM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	1 nM	n.p.
Incubation time of test substance	24 hours	16-24 hours
Antagonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	1 nM	n.p.
Incubation time of test substance	24 hours	n.p.

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Otsuka Pharmaceutical (2001)	**Ramamoorthy et al. (1997b)**
Characteristics of Cell Line		
Cell line	CHO	MDA-MB-231
Cell subtype	K1	
Cell source	Chinese hamster ovary	human breast cancer (ER-neg)
Transfection of Cells with Plasmids		
Transfection method/reagent	n.p.	Calcium phosphate
ER expression vector	pcDNA ERα	hER (undefined)
ER source	n.p.	human
ER transfection	Stable	Transient
Reporter vector	pINDERE-15	pC3-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Stable	Transient
Other plasmids	none	none
Other plasmid transfection	n.a.	n.a.
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.a.	24 hours
Time from transient transfection to treatment of cells	n.a.	18 hours
Stable transfection		
Plating time prior to treatment with test substance	24	n.a.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO
No. replicates	n.p.	n.p.
No. times assay repeated	n.p.	n.p.
Range of test substance concentrations	varies; about 1 pm to 100 nM	10 µM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	.001 µM	10 nM
Incubation time of test substance	16-24 hours	2 days
Antagonism	not done	not done
Reference ligand		
Concentration of reference ligand		
Incubation time of test substance		

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Ramamoorthy et al. (1997b)	**Rogers and Denison (2000)**
Characteristics of Cell Line		
Cell line	HepG2	BG-1
Cell subtype		BG1Luc4E2
Cell source	human liver cancer	human ovarian carcinoma
Transfection of Cells with Plasmids		
Transfection method/reagent	Lipofectinâ and OptiMEM	Polyprene
ER expression vector	hER (undefined)	endogenous
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	pC3-LUC	pGudLuc7ere
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	β-gal plasmid	none
Other plasmid transfection	Transient	n.a.
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	18 hours	n.p.
Time from transient transfection to treatment of cells	3 hours	48 hours
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO or ethanol
No. replicates	3	n.p.
No. times assay repeated	n.p.	At least 3
Range of test substance concentrations	1 nM to 1 µM	
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	n.p.	1 nM
Incubation time of test substance	24 hours	24 hours
Antagonism	not done	
Reference ligand		17β-Estradiol
Concentration of reference ligand		1 nM
Incubation time of test substance		24 hours

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Seinen et al. (1999)	**Seinen et al. (1999)**
Characteristics of Cell Line		
Cell line	HEK 293	HEK 293
Cell subtype		
Cell source	human embryonal kidney	human embryonal kidney
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	Calcium phosphate
ER expression vector	pSG5-HEGO (hERα)	pSG5-HEGO (hERα)
ER source	human	human
ER transfection	Transient	Stable
Reporter vector	3xERE-TATA-LUC	3xERE-TATA-LUC
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Stable
Other plasmids	SV2-LacZ	SV2-LacZ
Other plasmid transfection	Transient	Stable
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	24 hours	n.a.
Time from transient transfection to treatment of cells	16 hours	n.a.
Stable transfection		
Plating time prior to treatment with test substance	n.a.	48 hours
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	Ethanol	Ethanol
No. replicates	n.p.	n.p.
No. times assay repeated	n.p.	n.p.
Range of test substance concentrations	100 nM to 50 µM	100 nM to 50 µM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	0.1 pM to 1 nM	1 pM to 1 nM
Incubation time of test substance	24 hours	24 hours
Antagonism	not done	not done
Reference ligand		
Concentration of reference ligand		
Incubation time of test substance		

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Seinen et al. (1999)	**Seinen et al. (1999)**
Characteristics of Cell Line		
Cell line	HEK 293	HEK 293
Cell subtype		
Cell source	human embryonal kidney	human embryonal kidney
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	Calcium phosphate
ER expression vector	pSG5-hERβ	pSG5-hERβ
ER source	human	human
ER transfection	Transient	Stable
Reporter vector	3xERE-TATA-LUC	3xERE-TATA-LUC
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Stable
Other plasmids	SV2-LacZ	SV2-LacZ
Other plasmid transfection	Transient	Stable
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	24 hours	n.a.
Time from transient transfection to treatment of cells	16 hours	n.a.
Stable transfection		
Plating time prior to treatment with test substance	n.a.	48 hours
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	Ethanol	Ethanol
No. replicates	n.p.	n.p.
No. times assay repeated	n.p.	n.p.
Range of test substance concentrations	100 nM to 50 µM	100 nM to 50 µM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	0.1 nM to 100 nM	10 pM to 10 nM
Incubation time of test substance	24 hours	24 hours
Antagonism	not done	not done
Reference ligand		
Concentration of reference ligand		
Incubation time of test substance		

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Shelby et al. (1996)	**Sumida et al. (2001)**
Characteristics of Cell Line		
Cell line	HeLa	HeLa
Cell subtype		
Cell source	human cervical cancer	human cervical cancer
Transfection of Cells with Plasmids		
Transfection method/reagent	Electroporation	Lipofect AMINE™
ER expression vector	pRSV	pRc/RSV-hER
ER source	mouse	human
ER transfection	Transient	Transient
Reporter vector	ERET81CAT	pGV-tk-vEREx5
Reporter/endpoint	CAT expression	luciferase
Reporter transfection	Transient	Transient
Other plasmids		
Other plasmid transfection		
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.p.	n.p.
Time from transient transfection to treatment of cells	n.p.	Overnight
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional activation assay		
Metabolic activation	no	yes
Metabolic activation source	n.a.	S9 from induced rat liver
Test substance solvent	n.p.	DMSO
No. replicates	3	n.p.
No. times assay repeated	3	n.p.
Range of test substance concentrations	varies for substance; 0.01 to 10,000 nM	100 pM to 10 µM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	1 nM	n.p.
Incubation time of test substance	28 hours	24-28 hours
Antagonism	not done	not done
Reference ligand		
Concentration of reference ligand		
Incubation time of test substance		

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Sun et al. (1999)	**Sun et al. (1999)**
Characteristics of Cell Line		
Cell line	HEC-1	HEC-1
Cell subtype		
Cell source	human endometrial cancer	human endometrial cancer
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	Calcium phosphate
ER expression vector	pCMV5-hERα	pCMV5-hERβ
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	ERE3-pS2-CAT	ERE3-pS2-CAT
Reporter/endpoint	CAT	CAT
Reporter transfection	Transient	Transient
Other plasmids	pCH110 or pCMV-β-gal	pCH110 or pCMV-β-gal
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.p.	n.p.
Time from transient transfection to treatment of cells	n.p.	n.p.
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	n.p.	n.p.
No. replicates	n.p.	n.p.
No. times assay repeated	At least 3	At least 3
Range of test substance concentrations	1 pM to 1 µM	1 pM to 1 µM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	n.p.	n.p.
Incubation time of test substance	n.p.	n.p.
Antagonism	not done	
Reference ligand		17β-Estradiol
Concentration of reference ligand		1 nM
Incubation time of test substance		n.p.

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Sun et al. (1999)	**Sun et al. (1999)**
Characteristics of Cell Line		
Cell line	HEC-1	HEC-1
Cell subtype		
Cell source	human endometrial cancer	human endometrial cancer
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	Calcium phosphate
ER expression vector	pCMV5-hERα	pCMV5-hERβ
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	C3-Ti-LUC	C3-Ti-LUC
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pCH110 or pCMV-β-gal	pCH110 or pCMV-β-gal
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.p.	n.p.
Time from transient transfection to treatment of cells	n.p.	n.p.
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	n.p.	n.p.
No. replicates	n.p.	n.p.
No. times assay repeated	At least 3	At least 3
Range of test substance concentrations	1 pM to 1 µM	1 pM to 1 µM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	n.p.	n.p.
Incubation time of test substance	n.p.	n.p.
Antagonism	not done	
Reference ligand		17β-Estradiol
Concentration of reference ligand		1 nM
Incubation time of test substance		n.p.

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Tarumi et al. (2000)	**Tremblay et al. (1998)**
Characteristics of Cell Line		
Cell line	HeLa	COS-1
Cell subtype		
Cell source	human cervical cancer	monkey kidney
Transfection of Cells with Plasmids		
Transfection method/reagent	Lipofect AMINE™	Calcium phosphate
ER expression vector	hERα (undefined)	pCMX-mERα
ER source	human	mouse
ER transfection	Transient	Transient
Reporter vector	Luc (undefined)	vitA2-ERETKLuc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids		
Other plasmid transfection		
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	24 hours	n.p.
Time from transient transfection to treatment of cells	n.p.	8 to 16 hours
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	n.p.
No. replicates	6	n.p.
No. times assay repeated	n.p.	3
Range of test substance concentrations	varies for substance; 0.1-10 µg/mL or 10 nmol/L - 1 µmol/L	100 nM or 10 pM to 100 nM
Agonism		
Reference ligand	none	17β-Estradiol
Concentration of reference ligand	n.a.	n.p.
Incubation time of test substance	24 hours	16 hours
Antagonism	not done	
Reference ligand		17β-Estradiol
Concentration of reference ligand		10 nM
Incubation time of test substance		16 hours

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Tremblay et al. (1998)	**Tremblay et al. (1998)**
Characteristics of Cell Line		
Cell line	COS-1	COS-1
Cell subtype		
Cell source	monkey kidney	monkey kidney
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	Calcium phosphate
ER expression vector	pCMX-mERβ	pCMX-mERα
ER source	mouse	mouse
ER transfection	Transient	Transient
Reporter vector	vitA2-ERETKLuc	vitA2-EREBLuc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids		
Other plasmid transfection		
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.p.	n.p.
Time from transient transfection to treatment of cells	8 to 16 hours	8 to 16 hours
Stable transfection		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	n.p.	n.p.
No. replicates	n.p.	n.p.
No. times assay repeated	3	3
Range of test substance concentrations	100 nM or 10 pM to 100 nM	100 nM or 10 pM to 100 nM
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	n.p.	n.p.
Incubation time of test substance	16 hours	16 hours
Antagonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	10 nM	10 nM
Incubation time of test substance	16 hours	16 hours

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Tremblay et al. (1998)	Xenobiotic Laboratory Systems, Inc.
Characteristics of Cell Line		
Cell line	COS-1	BG-1
Cell subtype		BG1Luc4E2
Cell source	monkey kidney	human ovarian carcinoma
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	n.p.
ER expression vector	pCMX-mERβ	endogenous
ER source	mouse	human
ER transfection	Transient	n.a.
Reporter vector	vitA2-EREBLuc	pGudLuc7ere
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Stable
Other plasmids		
Other plasmid transfection		
Preparation of Cells for Assay		
Transient transfection		
Pregrowth of cells before transient transfection	n.p.	n.a.
Time from transient transfection to treatment of cells	8 to 16 hours	n.a.
Stable transfection		
Plating time prior to treatment with test substance	n.a.	24 hours
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	n.p.	DMSO
No. replicates	n.p.	1
No. times assay repeated	3	n.p.
Range of test substance concentrations	100 nM or 10 pM to 100 nM	10 µg/mL to 10 pg/mL
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Concentration of reference ligand	n.p.	n.p.
Incubation time of test substance	16 hours	24 hours
Antagonism		not done
Reference ligand	17β-Estradiol	
Concentration of reference ligand	10 nM	
Incubation time of test substance	16 hours	

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Zacherewski et al. (1998)
Characteristics of Cell Line	
Cell line	HeLa
Cell subtype	
Cell source	human
Transfection of Cells with Plasmids	
Transfection method/reagent	Calcium phosphate
ER expression vector	Gal4-HEGO (hERαdef)
ER source	human
ER transfection	Stable
Reporter vector	17m5-G-Luc
Reporter/endpoint	luciferase
Reporter transfection	Stable
Other plasmids	
Other plasmid transfection	
Preparation of Cells for Assay	
Transient transfection	
Pregrowth of cells before transient transfection	n.a.
Time from transient transfection to treatment of cells	n.a.
Stable transfection	
Plating time prior to treatment with test substance	24 hours
Transcriptional activation assay	
Metabolic activation	no
Metabolic activation source	n.a.
Test substance solvent	DMSO
No. replicates	2
No. times assay repeated	3
Range of test substance concentrations	100 nM to 10 µM
Agonism	
Reference ligand	17β-Estradiol
Concentration of reference ligand	1 pM - 10 nM
Incubation time of test substance	24 hours
Antagonism	not done
Reference ligand	
Concentration of reference ligand	
Incubation time of test substance	

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

Appendix A3

ER TA Cell Proliferation Assays

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ER Cell Proliferation Assays

	Arcaro et al. (1998)	**Arcaro et al. (1999a,b)**
Characteristics of Cell Line		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	n.p.	n.p.
Cell Proliferation Assay		
Test substance solvent	DMSO	DMSO
Range of test substance concentrations	maximum 5 µM	maximum 5 µM
No. replicates	4	4
No. times assay repeated	3	3
No. cells/well	$1x10^5$cells/mL/well	$1x10^5$cells/mL/well
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	n.p.	1.0 nM
Cell division/incubation	14 days	14 days
Measured as (e.g., cell division)	foci	foci
Antagonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	0.1 nM	1.0 nM
Cell division/incubation	14 days	14 days
Measured as (e.g., cell division)	foci	foci

Abbreviations: DMSO = dimethyl sulfoxide; n.a. = not applicable; n.p. = not provided

ER Cell Proliferation Assays

	Bonefeld-Jørgensen et al. (2001)	**Collins-Burow et al. (2000)**
Characteristics of Cell Line		
Cell line	MCF-7	MCF-7 (M variant p250)
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	24 hours	24 hours
Cell Proliferation Assay		
Test substance solvent	Ethanol	n.p.
Range of test substance concentrations	0.001 to 10 µM	100 nM, 25 µM
No. replicates	8	3
No. times assay repeated	3	At least 2
No. cells/well	10,000	50,000
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	10 pM	1.0 nM
Cell division/incubation	6 days	5 days
Measured as (e.g., cell division)	cell growth	cell growth
Antagonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	10 pM	1.0 nM
Cell division/incubation	n.p.	5 days
Measured as (e.g., cell division)	cell growth	cell growth

Abbreviations: DMSO = dimethyl sulfoxide; n.a. = not applicable; n.p. = not provided

ER Cell Proliferation Assays

	Dodge et al. (1996)	**Fielden et al. (1997)**
Characteristics of Cell Line		
Cell line	MCF-7 (ATCC HTB 22)	MCF-7
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	48 hours	n.p.
Cell Proliferation Assay		
Test substance solvent	DMSO	n.p.
Range of test substance concentrations	0.001 to 1000 nM	0.01-10 µM
No. replicates	3	3
No. times assay repeated	n.p.	2
No. cells/well	8000	n.p.
Agonism		
Reference ligand	17β-Estradiol	none
Final conc., reference ligand	n.p.	n.a.
Cell division/incubation	48 hours	n.p.
Measured as (e.g., cell division)	cell proliferation	cell growth
Antagonism	not done	
Reference ligand		17β-Estradiol
Final conc., reference ligand		1 nM
Cell division/incubation		n.p.
Measured as (e.g., cell division)		cell growth

Abbreviations: DMSO = dimethyl sulfoxide; n.a. = not applicable; n.p. = not provided

ER Cell Proliferation Assays

	Gierthy et al. (1997)	**Go et al. (1999)**
Characteristics of Cell Line		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	24 hours	24 hours
Cell Proliferation Assay		
Test substance solvent	n.p.	Ethanol
Range of test substance concentrations	50 nM to 5 µM	0.001 to 100 µM
No. replicates	4	2
No. times assay repeated	n.p.	3
No. cells/well	100,000	20,000
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	n.p.	10 nM
Cell division/incubation	14 days	6 days
Measured as (e.g., cell division)	colony formation	cell proliferation
Antagonism		not done
Reference ligand	17β-Estradiol	
Final conc., reference ligand	0.1 nM	
Cell division/incubation	14 days	
Measured as (e.g., cell division)	colony formation	

Abbreviations: DMSO = dimethyl sulfoxide; n.a. = not applicable; n.p. = not provided

ER Cell Proliferation Assays

	Harris et al. (1997)	**Harris et al. (1997)**
Characteristics of Cell Line		
Cell line	MCF-7	ZR-75
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	3-4 days	n.p.
Cell Proliferation Assay		
Test substance solvent	n.p.	n.p.
Range of test substance concentrations	10 µM	10 nM to 10 µM
No. replicates	2	2
No. times assay repeated	2	2
No. cells/well	n.p.	n.p.
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	10 nM	10 pM to 10 nM
Cell division/incubation	2, 5, 8, 12 days	11 days
Measured as (e.g., cell division)	cell division	cell division
Antagonism	not done	not done
Reference ligand		
Final conc., reference ligand		
Cell division/incubation		
Measured as (e.g., cell division)		

Abbreviations: DMSO = dimethyl sulfoxide; n.a. = not applicable; n.p. = not provided

ER Cell Proliferation Assays

	Ichikawa et al. (1997)	**Jobling et al. (1995)**
Characteristics of Cell Line		
Cell line	MCF-7	ZR-75
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	n.p.	n.p.
Cell Proliferation Assay		
Test substance solvent	DMSO	n.p.
Range of test substance concentrations	0.1 nM to 100 µM	10 µM
No. replicates	n.p.	2
No. times assay repeated	n.p.	2
No. cells/well	2000	n.p.
Agonism		
Reference ligand	none	17β-Estradiol
Final conc., reference ligand	n.a.	10 nM
Cell division/incubation	3 days	10 days
Measured as (e.g., cell division)	cell growth	cell growth
Antagonism	not done	not done
Reference ligand		
Final conc., reference ligand		
Cell division/incubation		
Measured as (e.g., cell division)		

Abbreviations: DMSO = dimethyl sulfoxide; n.a. = not applicable; n.p. = not provided

ER Cell Proliferation Assays

	Jones et al. (1998)	**Korner et al. (1995)**
Characteristics of Cell Line		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	Overnight to attach + 48 hours	24 hours
Cell Proliferation Assay		
Test substance solvent	n.p.	n.p.
Range of test substance concentrations	up to 0.1 µM	up to 1 mM
No. replicates	At least 3	At least 3
No. times assay repeated	n.p.	4
No. cells/well	10,000	10,000
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	1.0 nM	1 pM to 10 nM
Cell division/incubation	6 days	5 days
Measured as (e.g., cell division)	cell proliferation	Total protein content (SRB assay) or mitochondrial metabolic activity (MTT) as estimates of cell number
Antagonism	not done	not done
Reference ligand		
Final conc., reference ligand		
Cell division/incubation		
Measured as (e.g., cell division)		

Abbreviations: DMSO = dimethyl sulfoxide; n.a. = not applicable; n.p. = not provided

ER Cell Proliferation Assays

	Le Guevel and Pakdel (2001)	**Makela et al. (1994)**
Characteristics of Cell Line		
Cell line	Ishikawa	MCF-7
Cell source	human endometrial tumor	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	About 1 day	2 days
Cell Proliferation Assay		
Test substance solvent	Ethanol	Ethanol
Range of test substance concentrations	varies by substance (about 10^{-12}-10^{-7} mol/L)	10 pM to 1 µM
No. replicates	n.p.	n.p.
No. times assay repeated	6	8 - 12
No. cells/well	20,000 cells/200 µL	2000
Agonism		
Reference ligand	17α-Ethinyl estradiol	17β-Estradiol
Final conc., reference ligand	10^{-13} to 10^{-10} mol/L	1 nM
Cell division/incubation	48 hours	7 days
Measured as (e.g., cell division)	Alkaline phosphatase activity	cell growth
Antagonism	not done	
Reference ligand		17β-Estradiol
Final conc., reference ligand		1 nM
Cell division/incubation		n.p.
Measured as (e.g., cell division)		cell growth

Abbreviations: DMSO = dimethyl sulfoxide; n.a. = not applicable; n.p. = not provided

ER Cell Proliferation Assays

	Mellanen et al. (1996)	**Mellanen et al. (1996)**
Characteristics of Cell Line		
Cell line	MCF-7	T47D
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	About 1 day	About 1 day
Cell Proliferation Assay		
Test substance solvent	Ethanol	Ethanol
Range of test substance concentrations	1.0 pM to 1 µM	1.0 fM to 1.0 µM
No. replicates	3	3
No. times assay repeated	n.p.	n.p.
No. cells/well	2000	10,000
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	1 nM	1 nM
Cell division/incubation	7 days	10 days
Measured as (e.g., cell division)	cell proliferation	cell proliferation
Antagonism	not done	not done
Reference ligand		
Final conc., reference ligand		
Cell division/incubation		
Measured as (e.g., cell division)		

Abbreviations: DMSO = dimethyl sulfoxide; n.a. = not applicable; n.p. = not provided

ER Cell Proliferation Assays

	Miksicek (1993)	**Miodini et al. (1999)**
Characteristics of Cell Line		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	n.p.	24 hours
Cell Proliferation Assay		
Test substance solvent	n.p.	n.p.
Range of test substance concentrations	n.p.	0.5 - 20 µM
No. replicates	n.p.	4
No. times assay repeated	n.p.	3
No. cells/well	$5x10^3$ cell/cm^2	15,000
Agonism		
Reference ligand	17β-Estradiol	none
Final conc., reference ligand	10 nM	
Cell division/incubation	1 week	6 days
Measured as (e.g., cell division)	cell growth	cell growth
Antagonism	not done	
Reference ligand		17β-Estradiol
Final conc., reference ligand		0.01 µM
Cell division/incubation		7 hours
Measured as (e.g., cell division)		cell growth

Abbreviations: DMSO = dimethyl sulfoxide; n.a. = not applicable; n.p. = not provided

ER Cell Proliferation Assays

	Moore et al. (1997)	**Morito et al. (2001)**
Characteristics of Cell Line		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	24 hours	24 hours
Cell Proliferation Assay		
Test substance solvent	DMSO	n.p.
Range of test substance concentrations	0 -10 µM	n.p.
No. replicates	3	n.p.
No. times assay repeated	n.p.	n.p.
No. cells/well	50,000	20,000
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	1 nM	1 pM to 1 nM
Cell division/incubation	6 days	5 days
Measured as (e.g., cell division)	cell growth	cell growth
Antagonism		not done
Reference ligand	17β-Estradiol	
Final conc., reference ligand	1 nM	
Cell division/incubation	6 days	
Measured as (e.g., cell division)	cell growth	

Abbreviations: DMSO = dimethyl sulfoxide; n.a. = not applicable; n.p. = not provided

ER Cell Proliferation Assays

	Nakagawa & Suzuki (2001)	**Otsuka Pharmaceutical Co. (2001)**
Characteristics of Cell Line		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	24 hours	n.p.
Cell Proliferation Assay		
Test substance solvent	n.p.	n.p.
Range of test substance concentrations	1 nM to 500 µM	n.p.
No. replicates	3 or 4	n.p.
No. times assay repeated	n.p.	n.p.
No. cells/well	4000	n.p.
Agonism		n.p.
Reference ligand	17β-Estradiol	n.p.
Final conc., reference ligand	1 nM	n.p.
Cell division/incubation	5 days	n.p.
Measured as (e.g., cell division)	cell proliferation	cell proliferation
Antagonism	not done	not done
Reference ligand		
Final conc., reference ligand		
Cell division/incubation		
Measured as (e.g., cell division)		

Abbreviations: DMSO = dimethyl sulfoxide; n.a. = not applicable; n.p. = not provided

ER Cell Proliferation Assays

	Payne et al. (2001)	**Ramamoorthy et al. (1997a)**
Characteristics of Cell Line		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	24 hours after seeding + 72 hours	24 hours
Cell Proliferation Assay		
Test substance solvent	Ethanol	n.p.
Range of test substance concentrations	0.1 to 10 µM	100 nM to 10 µM
No. replicates	3	3
No. times assay repeated	2	n.p.
No. cells/well	10,000	50,000
Agonism		
Reference ligand	none	17β-Estradiol
Final conc., reference ligand	n.a.	1 nM
Cell division/incubation	7 days	11 days
Measured as (e.g., cell division)	cell proliferation	cell growth
Antagonism	not done	not done
Reference ligand		
Final conc., reference ligand		
Cell division/incubation		
Measured as (e.g., cell division)		

Abbreviations: DMSO = dimethyl sulfoxide; n.a. = not applicable; n.p. = not provided

ER Cell Proliferation Assays

	Schafer et al. (1999)	**Schafer et al. (1999)**
Characteristics of Cell Line		
Cell line	MCF-7 (subline BUS)	T47D
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	Overnight to 3 days	Overnight to 3 days
Cell Proliferation Assay		
Test substance solvent	Ethanol	Ethanol
Range of test substance concentrations	1 nM to 5 µM	1 nM to 5 µM
No. replicates	Higher doses: 4	Higher doses: 4
No. times assay repeated	n.p.	n.p.
No. cells/well	10,000	10,000
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	1 nM	1 nM
Cell division/incubation	6 to 7 days	6 to 7 days
Measured as (e.g., cell division)	cell proliferation	cell proliferation
Antagonism	not done	not done
Reference ligand		
Final conc., reference ligand		
Cell division/incubation		
Measured as (e.g., cell division)		

Abbreviations: DMSO = dimethyl sulfoxide; n.a. = not applicable; n.p. = not provided

ER Cell Proliferation Assays

	Schafer et al. (1999)	**Schlumpf et al. (2001)**
Characteristics of Cell Line		
Cell line	ZR-75-1	MCF-7 (Bos)
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	Overnight to 3 days	24 hours
Cell Proliferation Assay		
Test substance solvent	Ethanol	Ethanol
Range of test substance concentrations	1 nM to 5 µM	100 nM to 100 µM
No. replicates	Higher doses: 4	4
No. times assay repeated	n.p.	4 to 13
No. cells/well	10,000	40,000
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	1 nM	0.1 pM to 10 nM
Cell division/incubation	6 to 7 days	6 days
Measured as (e.g., cell division)	cell proliferation	cell proliferation
Antagonism	not done	
Reference ligand		17β-Estradiol
Final conc., reference ligand		10 pM
Cell division/incubation		6 days
Measured as (e.g., cell division)		cell proliferation

Abbreviations: DMSO = dimethyl sulfoxide; n.a. = not applicable; n.p. = not provided

ER Cell Proliferation Assays

	Soto et al. (1994)	**Soto et al. (1995)**
Characteristics of Cell Line		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	24 hours	24 hours
Cell Proliferation Assay		
Test substance solvent	Ethanol or DMSO	n.p.
Range of test substance concentrations	1 nM, 10 µM	100 nM to50 uM
No. replicates	2	2
No. times assay repeated	At least 5	At least 5
No. cells/well	20,000	20,000
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	1.0 pM to 10 nM	10 or 30 pM
Cell division/incubation	6 days	6 days
Measured as (e.g., cell division)	cell proliferation (relative proliferative potency & relative proliferative effect)	cell proliferation (relative proliferative potency & relative proliferative effect)
Antagonism	not done	not done
Reference ligand		
Final conc., reference ligand		
Cell division/incubation		
Measured as (e.g., cell division)		

Abbreviations: DMSO = dimethyl sulfoxide; n.a. = not applicable; n.p. = not provided

ER Cell Proliferation Assays

	Tamir et al. (2000)	**Tamir et al. (2000)**
Characteristics of Cell Line		
Cell line	T47D	MCF-7
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	48 hours	n.p.
Cell Proliferation Assay		
Test substance solvent	Ethanol	n.p.
Range of test substance concentrations	0.1 nM to 25 µM	1, 10, 25 µM
No. replicates	n.p.	n.p.
No. times assay repeated	3 or more	n.p.
No. cells/well	n.p.	n.p.
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	100 pM	10 nM
Cell division/incubation	7 days	3 weeks
Measured as (e.g., cell division)	cell proliferation	colony formation
Antagonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	100 pM	1 nM and 10 nM
Cell division/incubation	7 days	3 weeks
Measured as (e.g., cell division)	cell proliferation	colony formation

Abbreviations: DMSO = dimethyl sulfoxide; n.a. = not applicable; n.p. = not provided

ER Cell Proliferation Assays

	Vinggaard et al. (1999)	**Wiese et al. (1997)**
Characteristics of Cell Line		
Cell line	MCF-7 (E3 clone)	MCF-7 (E3 clone)
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	5 days	n.p.
Cell Proliferation Assay		
Test substance solvent	Ethanol	n.p.
Range of test substance concentrations	0.001, 0.1, 1, and 10 µM	1 pM to 1 µM
No. replicates	At least 3	3
No. times assay repeated	1, 2, 3, 4, 5, or 8 times	n.p.
No. cells/well	15,000	20,000
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	0.01 nM	10 pM
Cell division/incubation	up to 9 days	6 days
Measured as (e.g., cell division)	cell proliferation	cell growth
Antagonism		not done
Reference ligand	17β-Estradiol	
Final conc., reference ligand	0.01 nM	
Cell division/incubation	n.p.	
Measured as (e.g., cell division)	cell proliferation	

Abbreviations: DMSO = dimethyl sulfoxide; n.a. = not applicable; n.p. = not provided

Appendix A4

ER TA Reporter Gene Assays Using Yeast Cells

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ER TA Reporter Gene Assays Using Yeast Cells

	Arnold et al. (1996)	**Beresford et al. (2000)**
Characteristics of Yeast		
Species	*S. cerevisiae**	*S. cerevisiae**
Strain	BJ2407	n.p.
Stable Transfection of Cells with Plasmids		
ER expression vector	PSCW231-hER	hERα-ppk
ER source	human	human
Reporter vector	YRPE2 LacZ	vit2ERE-LacZ
Endpoint measured	β-galactosidase	β-galactosidase
Other plasmids	none	none
Transfection reagent	n.p.	n.p.
Plating time prior to treatment with test substance	n.p.	n.p.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	ethanol
Range of test substance concentrations	0.001 to 10,000 nM	n.p.
No. replicates	n.p.	n.p.
No. times assay repeated	n.p.	At least 2
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	1000 nM	4.88 pmoles - 2 nmoles
Incubation time of test substance	overnight	3 days
Measured as (e.g., color change, growth)	ONPG color change	CPRG color change
Antagonism	not done	
Reference ligand		17β-Estradiol
Final conc., reference ligand		2.5×10^{-10} M
Measured as (e.g., color change)		CPRG color change

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.
* Species name not provided in publication,
but likely *S. cerevisiae*

ER TA Reporter Gene Assays Using Yeast Cells

	Chen et al. (1997)	**Chen et al. (1997)**
Characteristics of Yeast		
Species	*S. cerevisiae**	*S. cerevisiae**
Strain	939	CTY10-5d
Stable Transfection of Cells with Plasmids		
ER expression vector	pUC19hER	LexA-hER
ER source	human	human
Reporter vector	YRpE2; cyc promotor; ERE-cyc-LacZ	ERE-LacZ
Endpoint measured	β-galactosidase	β-galactosidase
Other plasmids	none	none
Transfection reagent	n.p.	n.p.
Plating time prior to treatment with test substance	n.p.	n.p.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO
Range of test substance concentrations	n.p.	n.p.
No. replicates	3-6	3-6
No. times assay repeated	n.p.	n.p.
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	100 nM	100 nM
Incubation time of test substance	12 hours	12 hours
Measured as (e.g., color change, growth)	ONPG color change	ONPG color change
Antagonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	0.5 nM	1 nM
Measured as (e.g., color change)	ONPG color change	ONPG color change

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.
* Species name not provided in publication, but likely *S. cerevisiae*

ER TA Reporter Gene Assays Using Yeast Cells

	Coldham et al. (1997)	**Connor et al. (1996)**
Characteristics of Yeast		
Species	*S. cerevisiae**	*S. cerevisiae*
Strain	n.p.	PL3
Stable Transfection of Cells with Plasmids		
ER expression vector	ER-CUP1 MET	YEp10-HEGO
ER source	human	human
Reporter vector	2FR.vit-iso1-cytC-lacZ	URA 3
Endpoint measured	β-galactosidase	growth
Other plasmids	none	none
Transfection reagent	n.p.	Lithium acetate
Plating time prior to treatment with test substance	n.p.	n.p.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	n.p.	DMSO
Range of test substance concentrations	n.p.	n.p.
No. replicates	n.p.	4
No. times assay repeated	n.p.	3
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	n.p.	1 nM
Incubation time of test substance	18 hours	n.p.
Measured as (e.g., color change, growth)	ONPG color change	growth/no growth
Antagonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	0.001 - 10 µM	1 nM
Measured as (e.g., color change)	β-gal induction	cell division

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.
* Species name not provided in publication,
but likely *S. cerevisiae*

ER TA Reporter Gene Assays Using Yeast Cells

	De Boever et al. (2001)	**Elsby et al. (2001)**
Characteristics of Yeast		
Species	*S. cerevisiae*	*S. cerevisiae*
Strain	n.p.	n.p.
Stable Transfection of Cells with Plasmids		
ER expression vector	ERα (otherwise undefined)	ERα (otherwise undefined)
ER source	human	human
Reporter vector	n.p.	Yeast 3 phosphoglycerol promoter with EREs
Endpoint measured	β-galactosidase	β-galactosidase
Other plasmids	none	none
Transfection reagent	n.p.	n.p.
Plating time prior to treatment with test substance	Overnight	n.p.
Transcriptional activation assay		
Metabolic activation	yes	yes (2 chems) and no
Metabolic activation source	intestinal bacterial β-glycosidase	female Wistar rat liver; female human liver microsomes
Test substance solvent	ethanol	n.p.
Range of test substance concentrations	10^{-4} to 10^{-2} mol/L	1 pM to 100 nM
No. replicates	24 hours: 4 / 3-day: 4-6	2
No. times assay repeated	n.p.	4-8
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	6.96 nmoles	n.p.
Incubation time of test substance	24 hours / 3 days	3-4 days
Measured as (e.g., color change, growth)	CPRG color change	n.p.
Antagonism	not done	
Reference ligand		17β-Estradiol
Final conc., reference ligand		$1x10^{-10}$ M
Measured as (e.g., color change)		CPRG color change

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.
* Species name not provided in publication,
but likely *S. cerevisiae*

ER TA Reporter Gene Assays Using Yeast Cells

	Gaido et al. (1997)	**Grauman et al. (1999)**
Characteristics of Yeast		
Species	*S. cerevisiae*	*S. cerevisiae**
Strain	BJ3505	188R1
Stable Transfection of Cells with Plasmids		
ER expression vector	CUP1 hER	YEpE12 (HER) + CUP1
ER source	human	human
Reporter vector	ERE-LacZ	YRpE2 (vitellogenin A2ERE; cyc1; lacZ)
Endpoint measured	β-galactosidase	β-galactosidase
Other plasmids	CUP1 metallothionein promoter	CUP1 promotor
Transfection reagent	n.p.	n.p.
Plating time prior to treatment with test substance	n.p.	n.p.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	methanol	DMSO
Range of test substance concentrations	n.p.	10 pM to 100 nM
No. replicates	n.p.	n.p.
No. times assay repeated	3-5	n.p.
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	n.p.	varied
Incubation time of test substance	overnight	4 hours
Measured as (e.g., color change, growth)	ONPG color change	β-galactosidase activity
Antagonism	not done	
Reference ligand		17β-Estradiol
Final conc., reference ligand		n.p.
Measured as (e.g., color change)		β-galactosidase activity

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.
* Species name not provided in publication,
but likely *S. cerevisiae*

ER TA Reporter Gene Assays Using Yeast Cells

	Harris et al. (1997)	**Klotz et al. (1996)**
Characteristics of Yeast		
Species	*S. cerevisiae*	*S. cerevisiae**
Strain	n.p.	BJ2407
Stable Transfection of Cells with Plasmids		
ER expression vector	n.p.	PSCW231-hER
ER source	human	human
Reporter vector	vit2ERE-LacZ	YRP2ERE
Endpoint measured	β-galactosidase	β-galactosidase
Other plasmids	none	none
Transfection reagent	n.p.	n.p.
Plating time prior to treatment with test substance	Overnight	Overnight
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	ethanol	DMSO
Range of test substance concentrations	n.p.	n.p.
No. replicates	n.p.	3
No. times assay repeated	n.p.	2
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	1×10^{-8} M	0.01 µM
Incubation time of test substance	4-6 days	overnight
Measured as (e.g., color change, growth)	CPRG color change	ONPG color change
Antagonism	not done	not done
Reference ligand		
Final conc., reference ligand		
Measured as (e.g., color change)		

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.
* Species name not provided in publication,
but likely *S. cerevisiae*

ER TA Reporter Gene Assays Using Yeast Cells

	Lascombe et al. (2000)	**Le Guevel and Pakdel (2001)**
Characteristics of Yeast		
Species	*S. cerevisiae**	*S. cerevisiae*
Strain	YRG-2	BJ-ECZ
Stable Transfection of Cells with Plasmids		
ER expression vector	p2HG-hER	hER (otherwise undefined)
ER source	human	human
Reporter vector	pLGERE-CYC-1	ERE CYC1
Endpoint measured	β-galactosidase	β-galactosidase
Other plasmids	none	none
Transfection reagent	n.p.	n.p.
Plating time prior to treatment with test substance	Overnight	36 hours
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	Ethanol	DMSO
Range of test substance concentrations	10 pM to 1 µM	10^{-10} to 10^{-5} mol/L
No. replicates	3	4
No. times assay repeated	At least 2	6-9
Agonism		
Reference ligand	17β-Estradiol	Ethinyl estradiol
Final conc., reference ligand	n.p.	n.p.
Incubation time of test substance	overnight	4 hours
Measured as (e.g., color change, growth)	β-gal induction	ONPG color change
Antagonism		not done
Reference ligand	17β-Estradiol	
Final conc., reference ligand	n.p.	
Measured as (e.g., color change)	β-gal induction	

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.
* Species name not provided in publication, but likely *S. cerevisiae*

ER TA Reporter Gene Assays Using Yeast Cells

	Le Guevel and Pakdel (2001)	**Miller et al. (2001)**
Characteristics of Yeast		
Species	*S. cerevisiae*	*S. cerevisiae**
Strain	BJ-ECZ	n.p.
Stable Transfection of Cells with Plasmids		
ER expression vector	rtER (otherwise undefined)	ERα (otherwise undefined)
ER source	rainbow trout	human
Reporter vector	ERE2-CYC1-LacZ	"expression plasmids" with EREs ppk
Endpoint measured	β-galactosidase	β-galactosidase
Other plasmids	none	none
Transfection reagent	n.p.	n.p.
Plating time prior to treatment with test substance	36 hours	n.p.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	ethanol
Range of test substance concentrations	10^{-10} to 10^{-5} mol/L	1 pM to 1 mM
No. replicates	4	n.p.
No. times assay repeated	6-12	At least 2
Agonism		
Reference ligand	Ethinyl estradiol	17β-Estradiol
Final conc., reference ligand	n.p.	4.88 pmoles - 2 nmoles
Incubation time of test substance	4 hours	3 days
Measured as (e.g., color change, growth)	ONPG color change	CPRG color change
Antagonism	not done	not done
Reference ligand		
Final conc., reference ligand		
Measured as (e.g., color change)		

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.
* Species name not provided in publication,
but likely *S. cerevisiae*

ER TA Reporter Gene Assays Using Yeast Cells

	Moffat et al. (2001)	Morito et al. (2001a)
Characteristics of Yeast		
Species	*S. cerevisiae*	*S. cerevisiae*
Strain	n.p.	Y190
Stable Transfection of Cells with Plasmids		
ER expression vector	hER (otherwise undefined)	pGBT9-hERα
ER source	human	human
Reporter vector	n.p.	n.p.
Endpoint measured	β-galactosidase	β-galactosidase
Other plasmids	none	none
Transfection reagent	n.p.	n.p.
Plating time prior to treatment with test substance	n.p.	n.p.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	Ethanol	n.p.
Range of test substance concentrations	10^{-12} to 0.1 M	0.01 pM to 0.1 mM
No. replicates	n.p.	n.p.
No. times assay repeated	n.p.	n.p.
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	n.p.	n.p.
Incubation time of test substance	3 days	n.p.
Measured as (e.g., color change, growth)	β-gal induction	β-gal induction
Antagonism		not done
Reference ligand	17β-Estradiol	
Final conc., reference ligand	0.005 nM	
Measured as (e.g., color change)	β-gal induction	

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.
* Species name not provided in publication,
but likely *S. cerevisiae*

ER TA Reporter Gene Assays Using Yeast Cells

	Morito et al. (2001a)	**Morito et al. (2001b)**
Characteristics of Yeast		
Species	*S. cerevisiae*	*S. cerevisiae*
Strain	Y190	Y190
Stable Transfection of Cells with Plasmids		
ER expression vector	pGBT9-hERβ	pGBT9-hERα
ER source	human	human
Reporter vector	n.p.	n.p.
Endpoint measured	β-galactosidase	β-galactosidase
Other plasmids	none	none
Transfection reagent	n.p.	n.p.
Plating time prior to treatment with test substance	n.p.	n.p.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	n.p.	n.p.
Range of test substance concentrations	0.01 pM to 0.1 mM	1 pM to 0.1 mM
No. replicates	n.p.	n.p.
No. times assay repeated	n.p.	n.p.
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	n.p.	n.p.
Incubation time of test substance	n.p.	n.p.
Measured as (e.g., color change, growth)	β-gal induction	β-gal induction
Antagonism	not done	
Reference ligand		17β-Estradiol
Final conc., reference ligand		1 nM
Measured as (e.g., color change)		β-gal induction

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.
* Species name not provided in publication,
but likely *S. cerevisiae*

ER TA Reporter Gene Assays Using Yeast Cells

	Morito et al. (2001b)	**Odum et al. (1999)**
Characteristics of Yeast		
Species	*S. cerevisiae*	*S. cerevisiae*
Strain	Y190	n.p.
Stable Transfection of Cells with Plasmids		
ER expression vector	pGBT9-hERβ	hER
ER source	human	human
Reporter vector	n.p.	n.p.
Endpoint measured	β-galactosidase	β-galactosidase
Other plasmids	none	none
Transfection reagent	n.p.	n.p.
Plating time prior to treatment with test substance	n.p.	n.p.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	n.p.	ethanol
Range of test substance concentrations	1 pM to 0.1 mM	1 pM to 1mM
No. replicates	n.p.	n.p.
No. times assay repeated	n.p.	n.p.
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	n.p.	n.p.
Incubation time of test substance	n.p.	4 days
Measured as (e.g., color change, growth)	β-gal induction	color change (otherwise undef.)
Antagonism		not done
Reference ligand	17β-Estradiol	
Final conc., reference ligand	1 nM	
Measured as (e.g., color change)	β-gal induction	

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.
* Species name not provided in publication,
but likely *S. cerevisiae*

ER TA Reporter Gene Assays Using Yeast Cells

	Petit et al. (1997)	**Petit et al. (1999)**
Characteristics of Yeast		
Species	*S. cerevisiae*	*S. cerevisiae*
Strain	BJ-ECZ	BJ-ECZ
Stable Transfection of Cells with Plasmids		
ER expression vector	YEprtER	YEprtER
ER source	rainbow trout	rainbow trout
Reporter vector	2ERE-CYC1-LacZ	2ERE-CYC1-LacZ
Endpoint measured	β-galactosidase	β-galactosidase
Other plasmids	none	none
Transfection reagent	n.p.	n.p.
Plating time prior to treatment with test substance	n.p.	n.p.
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	ethanol or DMSO	ethanol or DMSO
Range of test substance concentrations	10 pM to 0.1 mM	10 pM to 0.1 mM
No. replicates	n.p.	n.p.
No. times assay repeated	At least 3	At least 2
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	10 nM	10 nM
Incubation time of test substance	4 hours	4 hours
Measured as (e.g., color change, growth)	β-gal induction	β-gal induction
Antagonism	not done	not done
Reference ligand		
Final conc., reference ligand		
Measured as (e.g., color change)		

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.
* Species name not provided in publication,
but likely *S. cerevisiae*

ER TA Reporter Gene Assays Using Yeast Cells

	Rajapakse et al. (2001)	Ramamoorthy et al. (1997a)
Characteristics of Yeast		
Species	*S. cerevisiae**	*S. cerevisiae**
Strain	n.p.	BJ2168
Stable Transfection of Cells with Plasmids		
ER expression vector	hER	CUP1
ER source	human	mouse
Reporter vector	n.p.	vit ERE1-lacZ
Endpoint measured	β-galactosidase	β-galactosidase
Other plasmids	none	none
Transfection reagent	n.p.	Lithium acetate
Plating time prior to treatment with test substance	Overnight	Overnight
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	Ethanol	DMSO
Range of test substance concentrations	10^{-5} to 10^{-2} µM	250 nM to 25 µM
No. replicates	n.p.	n.p.
No. times assay repeated	n.p.	4
Agonism		
Reference ligand	17β-Estradiol	Diethylstilbestrol
Final conc., reference ligand	n.p.	1 and 10 nM
Incubation time of test substance	72 hours	2.5 hours or 16 hours
Measured as (e.g., color change, growth)	ONPG color change	β-gal induction
Antagonism	not done	
Reference ligand		Diethylstilbestrol
Final conc., reference ligand		10 nM
Measured as (e.g., color change)		β-gal induction

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.
* Species name not provided in publication,
but likely *S. cerevisiae*

ER TA Reporter Gene Assays Using Yeast Cells

	Ramamoorthy et al. (1997a)	**Ramamoorthy et al. (1997b)**
Characteristics of Yeast		
Species	*S. cerevisiae**	*S. cerevisiae*
Strain	BJ2407	BJ3505
Stable Transfection of Cells with Plasmids		
ER expression vector	YePE10	CUP1-hER
ER source	human	human
Reporter vector	YRPE2 vit 2 CYC1	n.p.
Endpoint measured	β-galactosidase	β-galactosidase
Other plasmids	none	none
Transfection reagent	Lithium acetate	n.p.
Plating time prior to treatment with test substance	Overnight	Overnight
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO
Range of test substance concentrations	n.p.	0.1 pM to 10 µM
No. replicates	n.p.	3
No. times assay repeated	n.p.	n.p.
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	10 nM	.001 µM
Incubation time of test substance	24 hours	overnight
Measured as (e.g., color change, growth)	β-gal induction	ONPG color change
Antagonism		not done
Reference ligand	17β-Estradiol	
Final conc., reference ligand	10 nM	
Measured as (e.g., color change)	β-gal induction	

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.
* Species name not provided in publication,
but likely *S. cerevisiae*

ER TA Reporter Gene Assays Using Yeast Cells

	Routledge & Sumpter (1996)	**Routledge and Sumpter (1997)**
Characteristics of Yeast		
Species	*S. cerevisiae*	*S. cerevisiae*
Strain	n.p.	n.p.
Stable Transfection of Cells with Plasmids		
ER expression vector	hER	hER
ER source	human	human
Reporter vector	vit 2 β-gal	n.p.
Endpoint measured	β-galactosidase	β-galactosidase
Other plasmids	none	none
Transfection reagent	n.p.	n.p.
Plating time prior to treatment with test substance	24 hours	24 hours
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	ethanol	ethanol
Range of test substance concentrations	60 nM to 5 mM	1 pM to 10 mM
No. replicates	n.p.	n.p.
No. times assay repeated	n.p.	1
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	3000 ng/L	10 nM
Incubation time of test substance	3 days	3 days
Measured as (e.g., color change, growth)	CPRG color change	CPRG color change
Antagonism	not done	
Reference ligand		none
Final conc., reference ligand		n.p.
Measured as (e.g., color change)		CPRG color change

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.
* Species name not provided in publication,
but likely *S. cerevisiae*

ER TA Reporter Gene Assays Using Yeast Cells

	Tran et al. (1996)	**Tran et al. (1996)**
Characteristics of Yeast		
Species	*S. cerevisiae*	*S. cerevisiae*
Strain	ER (wt)	ER179C
Stable Transfection of Cells with Plasmids		
ER expression vector	hER	hER
ER source	human	human
Reporter vector	n.p.	n.p.
Endpoint measured	β-galactosidase	β-galactosidase
Other plasmids	none	none
Transfection reagent	n.p.	n.p.
Plating time prior to treatment with test substance	Overnight	Overnight
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO
Range of test substance concentrations	n.p.	n.p.
No. replicates	n.p.	n.p.
No. times assay repeated	n.p.	n.p.
Agonism		not done
Reference ligand	17β-Estradiol	
Final conc., reference ligand	0.5 nM	
Incubation time of test substance	12 hours	
Measured as (e.g., color change, growth)	growth; ONPG color change	
Antagonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	0.5 nM	0.5 nM
Measured as (e.g., color change)	ONPG color change	growth; ONPG color change

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.
* Species name not provided in publication,
but likely *S. cerevisiae*

ER TA Reporter Gene Assays Using Yeast Cells

	Vinggaard et al. (1999)	**Vinggaard et al. (2000)**
Characteristics of Yeast		
Species	*S. cerevisiae*	*S. cerevisiae*
Strain	n.p.	n.p.
Stable Transfection of Cells with Plasmids		
ER expression vector	hERα	hERα
ER source	human	human
Reporter vector	vit 2 β-gal	vit 2 β-gal
Endpoint measured	β-galactosidase	β-galactosidase
Other plasmids	none	none
Transfection reagent	n.p.	n.p.
Plating time prior to treatment with test substance	24 hours	24 hours
Transcriptional activation assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	ethanol	ethanol
Range of test substance concentrations	0.2 µM to 550 µM	0.2 µM to 550 µM
No. replicates	n.p.	n.p.
No. times assay repeated	3	2
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final conc., reference ligand	0.2 - 500 pM	0.24 - 500 pM
Incubation time of test substance	4 days	4 days
Measured as (e.g., color change, growth)	CPRG color change	CPRG color change
Antagonism	not done	not done
Reference ligand		
Final conc., reference ligand		
Measured as (e.g., color change)		

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.
* Species name not provided in publication, but likely *S. cerevisiae*

ER TA Reporter Gene Assays Using Yeast Cells

	Yoshihara et al. (2001)
Characteristics of Yeast	
Species	*S. cerevisiae**
Strain	n.p.
Stable Transfection of Cells with Plasmids	
ER expression vector	hERα
ER source	human
Reporter vector	lacZ
Endpoint measured	β-galactosidase
Other plasmids	none
Transfection reagent	n.p.
Plating time prior to treatment with test substance	n.p.
Transcriptional activation assay	
Metabolic activation	yes
Metabolic activation source	male Wistar rat
Test substance solvent	ethanol
Range of test substance concentrations	n.p.
No. replicates	At least 2
No. times assay repeated	n.p.
Agonism	
Reference ligand	17β-Estradiol
Final conc., reference ligand	0.05 µM
Incubation time of test substance	1 hours (+S9); 24-48 hours (-S9)
Measured as (e.g., color change, growth)	β-gal induction
Antagonism	not done
Reference ligand	
Final conc., reference ligand	
Measured as (e.g., color change)	

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.
* Species name not provided in publication, but likely *S. cerevisiae*

Appendix B

In Vitro ER TA Assay Protocols

B1 **Protocol for HepG2 Cells + Receptor + Reporter and/or β-gal Plasmids for Use in Steroid Hormone Receptor Assays**
(Provided by Dr. Kevin Gaido, CIIT Centers for Health Research, Research Triangle Park, NC, USA)

B2 **Protocol for Chimeric ERα-Mediated Reporter Gene Expression in MCF-7 Cells**
(Provided by Dr. Timothy Zacharewski, Dept. of Biochemistry, Michigan State University, Lansing, MI, USA)

B3 **Protocol for the Development of new reporter gene assay systems for screening Endocrine Disrupters**
(Provided by Drs. Mitsuru Iida and Teruhisa Kato, Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan)

B4 **Protocol for the Development of stably transfected cell lines to screen Endocrine Disrupters**
(Provided by Drs. Mitsuru Iida and Teruhisa Kato, Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan)

B5 **Technical Perspective on the U.S.E.P.A. Endocrine Disruptor Screening Program: In Vitro EDSTAC Guideline Protocols**
(Provided by Dr. Grantley Charles, Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, MI, USA, and Dr. William Kelce, Pharmacia Corporation, Kalamazoo, MI, USA)

B6 **Lyticase-based cell lysis protocol of β-Galactosidase assay for 96 well plates**
(Provided by Dr. Rémy Le Guével of the Université de Rennes, Rennes, France)

B7 **High-Throughput System for Screening Estrogen-Like Chemicals**
(Provided by Dr. George C. Clark, Xenobiotic Detection Systems, Durham, NC, USA)

B8 Protocol for the MVLN Assay
(Provided by Dr. Thomas E. Wiese, Division of Basic Pharmaceutical Sciences, Xavier University of Louisiana, and Dept. of Environmental Health Sciences, Tulane University, New Orleans, LA, USA

Appendix B1

Protocol for HepG2 Cells + Receptor + Reporter and/or β-gal plasmids for Use in Steroid Hormone Receptor Assays

(Provided by Dr. Kevin Gaido, CIIT Centers for Health Research, Research Triangle Park, NC, USA)

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TransIT Transfection Method of HepG2 Cells for Use in Steroid Hormone Receptor Assays

1. MATERIALS AND SOURCES:

a. TransIT-LT1 Transfection Reagent, supplier: Mirus Corporation, CAT. #: MIR 2300.
b. 1X Phosphate Buffered Saline Solution.
c. Plasmid DNA's of choice: i.e., receptor, reporter, and/or β- gal plasmids.
d. Phenol red-free Minimum Essential Medium (MEM).
e. Complete phenol red-free Minimum Essential Medium (MEM), with stripped (or charcoal/dextran treated) fetal bovine serum.
f. 0.02% EDTA.
g. Trypsin, 2.5%.
h. Dimethyl sulfoxide.
i. 1M Sodium pyruvate.
j. L-glutamine (100X).

2. EQUIPMENT AND SUPPLIES:

a. Incubator with 5% CO_2/air, 37°C
b. Vortexer
c. 10 µl, 100 µl, 200 µl, and 1000 µl Eppendorf pipettor or equivalent
d. pipet tips
e. 1, 2, 5, 10, 25, and 50 ml pipets
f. 500 ml screw cap glass bottles, sterile
g. 24 well tissue culture plates
h. 15 and 50 ml centrifuge tubes, sterile, polypropylene
i. 17x100, polypropylene snap-cap tubes, sterile, round bottom
j. 1.5 ml siliconized polypropylene screw-cap vials

3. PREPARATION:

a. **0.12% Trypsin/0.02% EDTA.**
In 500 ml sterile screw cap glass bottle, sterilely transfer 190 ml 0.02% EDTA. Add 10 ml of 2.5% trypsin. Store at 4°C.

b. **Complete phenol red-free MEM.**
To 500 ml of phenol red-free MEM, add 0.5 ml 1M sodium pyruvate solution, 10.0 ml glutamine, and 50 ml resin-stripped (or charcoal dextran treated) fetal bovine serum. Store 4°C.

c. **Chemicals.**
Dissolve chosen chemical to make a 0.1M stock solution using appropriate vehicle. Make serial dilutions in 1.5 ml polypropylene screw-cap vials to yield a standard curve of concentrations varying from 10^{-5}M to 10^{-11}M (may be changed as necessary).

4. PROCEDURE:

Plating Cells.

a. Aspirate medium from 150 mm plate of 75-80% confluent HepG2 cells and rinse with 10 ml of 0.02% EDTA.
b. Place 10 ml of 0.12% trypsin/0.02% EDTA on plate.
c. Place in incubator until cells begin to detach (~5 min).
d. After cells have detached, pipette vigorously to remove the cells and transfer to 50 ml polypropylene centrifuge tube containing complete phenol red-free MEM.
e. Rinse plate with complete phenol red-free MEM and add to tube.
f. Centrifuge at 1000 RPM for 5 min at 4°C.
g. Carefully aspirate supernatant and resuspend the pellet in phenol red-free complete MEM.
h. Take cell count. Plate cells in 24-well tissue culture dishes at 10^5 cells/0.5 ml complete phenol red-free MEM. Swirl the plate gently to spread cells evenly in wells.
i. Place cells in 37°C incubator with 5% CO_2/air for 18 hours.

Transfecting Cells.

In a 17x100 ml round bottom, polypropylene, snap cap tube, add the following reagents: (For transfection of a 24-well tissue culture plate)

a. 0.65 ml of phenol red-free MEM **without any additives.**
b. Appropriate amount of TransIT LT1 reagent. For every **µg** of DNA plasmid, add **2 µl** of TransIT LT1 reagent. (11 µl of TransIT LT-1 reagent is needed for the suggested amounts of plasmid listed in 3. Below.) Mix **very gently** and let sit at RT for at least 5 min.
c. Carefully add appropriate amounts of receptor, promoter, and β- gal plasmids. This may vary depending on the application. A suggestion for amounts is as follows:

Estrogen Assay Example

Receptor Plasmid: 7 ng/well
pCMVβ Plasmid (β-gal): 30 ng/well
Promoter Plasmid: 200 ng/well

a. Mix **very gently** and let sit at RT for at least 5 min.
b. To each well of the 24 well plate containing HepG2 cells, carefully add 25 µl of the TransIT/DNA complex.
c. Place plate in incubator and allow to incubate for 3 hr at 37°C.

Treating cells.

a. Dilute chosen chemicals 1:1000 in complete phenol red-free MEM, to create final concentrations ranging from 10^{-5} to 10^{-11} M (this may vary as necessary).

b. After the 3 hr incubation, aspirate the media and add 0.5 ml/well of the chemical diluted in media.

c. Return plate to incubator and incubate for 24 hr. Collect cell lysate for β-gal and luciferase assays.

Lysis Procedure

1. MATERIALS:

a. Phosphate Buffered Saline (1X PBS).
b. Tris base.
c. Trans-1, 2-diaminocyclohexane-N, N, N', N'-tetraacetic acid (CDTA).
d. Glycerol.
e. Phosphoric Acid.
f. Triton X-100.
g. 1M Dithiothreitol (DTT).
h. Transfected cells plated in 24-well plate.

2. EQUIPMENT AND SUPPLIES:

a. 1-200 µl Pipettor
b. Multi-channel pipettor,1-100 µl
c. 1-200 µl pipette tips
d. Pipette aid
e. Vacuum system with hazardous waste flask attached
f. pH meter
g. 5 3/4" Pasteur pipette
h. 500 ml squeeze water bottle
i. 96 well ELISA plate
j. 96 well Plate, white
k. 250 ml Glass beakers
l. 100 and 200 ml Graduated cylinders
m. Stirrer and stir bars

3. PREPARATION:

a. 5X Lysis Solution.

a. Weigh out 3.03g Tris Base and 0.695g CDTA and place in 250 ml beaker.
b. Dissolve completely in 60 ml of dH_2O.
c. Measure 100 ml glycerol in 100 ml graduated cylinder, pour into fresh 250 ml beaker.
d. Rinse 100 ml cylinder with Tris base/CDTA. Add to glycerol in 250 ml beaker. Mix well.
e. pH to 7.8 with phosphoric acid (H_3PO_4) if necessary.
f. Add dH_2O to 200 ml.
g. Add 5 ml of 100% Triton X-100 (solution will look cloudy/milky). Store room temperature.

b. 1X Lysis Solution.

In 50 ml centrifuge tube, dilute 5X Lysis Solution to 1X by diluting 1 ml 5X lysis solution into 4 ml dH_2O. Add **30 µl** 1M DTT per **10 ml** 1X lysis solution. Make fresh each time. Make up enough 1X lysis solution to dispense 65µl per well.

4. PROCEDURE:

a. Aspirate media from wells and rinse with ≈0.5ml of PBS per well.

b. Aspirate PBS from wells and with multi-channel pipettor; dispense 65 µl of 1X lysis solution per well.

c. Let sit at room temperature for 20 min, rocking occasionally.

d. Transfer 30 µl of cell lysate to 96 well ELISA plate. This will be used for the β-galactosidase assay.

e. Transfer 20 µl of cell lysate to a 96 well white plate. This will be used for the luciferase assay.

ß-Galactosidase Assay Using Chlorophenol Red-β-D-galactopyranoside

1. MATERIALS AND SOURCES:

a. Chlorophenol red-ß-D-galactopyranoside (CPRG).
b. Disodium phosphate ($Na_2HPO_4 \cdot 7H_20$).
c. Monosodium phosphate ($NaH_2PO_4 \cdot H_20$).
d. Potassium chloride (KCl).
e. Magnesium sulfate ($MgSO_4 \cdot 7H_2O$).
f. β-Mercaptoethanol (2-ME).

2. EQUIPMENT AND SUPPLIES:

a. Spectrophotmetric microplate reader, with a 575 nm filter and kinetics capability
b. Multi-channel pipettor
c. Graduated cylinder, 1000 ml
d. Balance
e. Stir plate
f. Magnetic stir bar
g. 1-100 µl pipettor
h. 1-100 µl pipet tips
i. Pipettor reservoirs
j. 0.2 µ Filter unit
k. 96 well ELISA plate
l. 1 L beaker
m. 50 ml centrifuge tube, polypropylene, sterile

3. CPRG BUFFER PREPARATION:

a. Weigh out in 1 L beaker:
b. 16.1 g $Na_2HPO_4 \cdot 7H_20$
c. 5.5 g $NaH_2PO_4 \cdot H_20$
d. 0.75 g KCl
e. 0.25 g $MgSO_4 \cdot 7H_2O$
f. Dissolve in 800 ml of distilled water with stirring.
g. Adjust pH to 7.8.
h. Transfer to 1000 ml graduated cylinder. Bring up to 1000 ml with distilled water.
i. Filter sterilize. Store at room temperature.

4. ASSAY PROCEDURE:

a. Pipet 30 µl of cell lysate into a 96 well plate (usually done in triplicate).

b. **PER WELL OF 96 WELL PLATE**, add 170 µl of CPRG reagent made up as follows: 80 µg CPRG dissolved in 20 µl distilled water, 150 µl of CPRG buffer, and 0.84 µl 2-ME (1/200 dilution).

c. Using multi-channel pipettor, dispense 170 µl of CPRG reagent into each well containing lysate. For plate blank, use 30 µl of lysis solution and add 170 µl of CPRG reagent.

Set spectrophotometer microplate reader to kinetic endpoint and read the plate at 575 nm at 1 min intervals for 30 min to obtain Vmax. Samples will change from yellow to dark red as reaction occurs.

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Appendix B2

Protocol for Chimeric ERα-Mediated Reporter Gene Expression in MCF-7 Cells

(Provided by Dr. Timothy Zacharewski, Dept. of Biochemistry, Michigan State University, Lansing, MI, USA)

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TRANSCRIPTIONAL ACTIVATION: CHIMERIC ERα-MEDIATED REPORTER GENE EXPRESSION IN MCF-7 CELLS

Thawing of cells:

1. Remove vial of cells from liquid nitrogen tank and thaw with hands.
2. Add cells to 10ml of prewarmed 10% fetal bovine serum (FBS; Intergen) in Dulbecco's Modified Eagle's Medium (DMEM; Gibco) and incubate at 37C.
3. Change media after approximately 24hrs, then pass cells (see below) at confluence, after approximately another 30hrs, to one T-75 flask in 15ml DMEM/10% FBS. At confluence (approximately 15 million cells) split the T-75 to two T-75s, then the two T-75s to five T-75s. Cells can then be maintained by splitting each T-75 to three new T-75s every three days. For assays one confluent T-75 flask can be used for 5 6-well plates (~500,000 cells per well).

Note: cells are used for transfection assays until their responsiveness diminishes noticeably. We have found this to be after approximately 10 passages, but this will vary depending on the initial passage number of the cells.

Passage of cells:

1. Aspirate off media into collection flask containing bleach.
2. Add approximately 2ml prewarmed trypsin to the T-75 flask (1ml to a T-25 flask) and briefly rinse the cells. Aspirate off the trypsin.
3. Add 2ml fresh trypsin (1ml to a T-25 flask) to rinsed cells and incubate at 37C for approximately 5min.
4. Firmly tap the flask enough to dislodge the cells from the inner surface of the flask. Verify under a microscope that the cells free of the surface and floating singly.
5. Add approximately 10ml DMEM/10% FBS (<5ml for a T-25 flask) and transfer the media and cells to a 50ml sterile disposable centrifuge tube.
6. Centrifuge at 500g for 1min.
7. Carefully remove media/trypsin.
8. Resuspend in an appropriate volume of media: 15ml DMEM/10% FBS per T-75 for maintenance, or 12ml DMEM/5% FBS-DCC (see recipe) per 6-well plate for transfection assays. Initially add a small volume (~5ml) and gently breaking up clumps of cells by repeatedly resuspending with a Pasteur pipette before adding the rest of the media and then mixing thoroughly.

Note: for transfection assays, an extra well (single 35mm Petri dish) is also plated as a blank, which is not transfected or dosed.

Transient transfection assay:

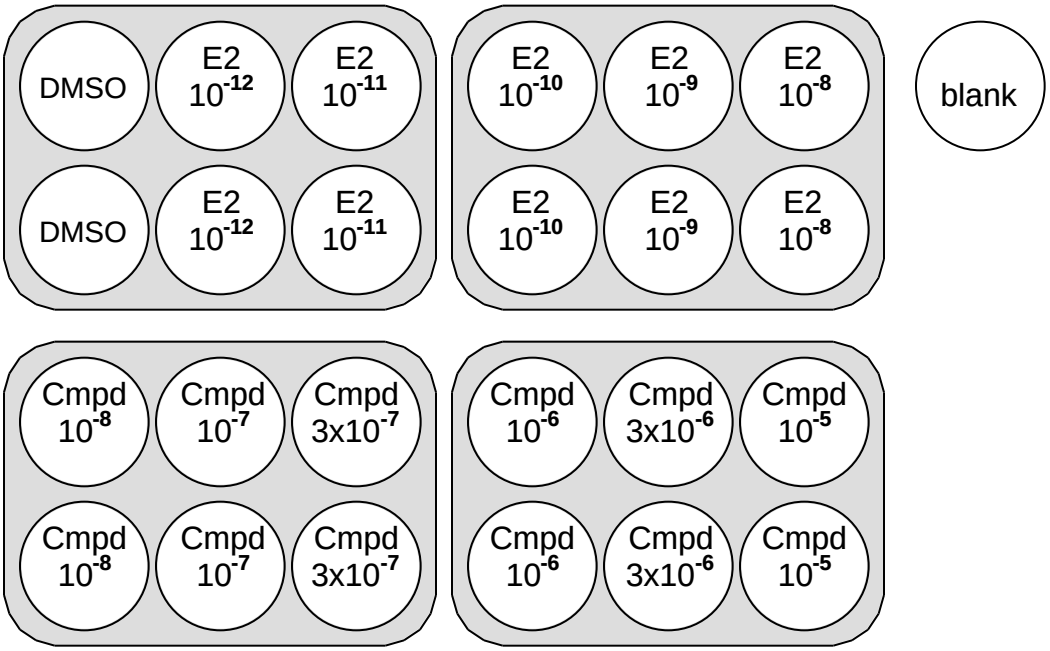
Cells split into 6-well plates in DMEM/5% FBS-DCC are incubated 7hrs at 37C to allow attachment to the plate surface. Transfection is typically performed by the calcium phosphate coprecipitation method outlined in Molecular Cloning (Sambrook, Fritsch, and Maniatis) and summarized below; an alternative effective method follows the Lipofectamine reagent protocol (Gibco).

1. Add appropriate amounts of maxiprepped DNA (0.2µg/well Gal4-ERα def; 1.5µg /well 17m5-G-Luc; 0.15µg /well pCMV-lacZ) to 100ul/well TE (see recipe) in a sterile tube.
2. Add 15µl/well 2M $CaCl_2$ (see recipe) dropwise to the DNA/TE mixture with gentle vortexing.
3. Add the DNA/TE/ $CaCl_2$ solution dropwise to 120µl/well 2xHBS (see recipe) with gentle vortexing.
4. Incubate 20min at room temperature.
5. Add 235µl of the mixture dropwise to each well of each 6-well plate.

6. Incubate at 37C for 16hr.

Dosing of cells:

1. Rinse cells twice with prewarmed sterile phosphate-buffered saline (PBS).
2. Aspirate off PBS and add 2ml DMEM/5% FBS-DCC.
3. Dose cells by adding 2µl of test compound dissolved in appropriate solvent (often dimethyl sulfoxide) at various concentrations in duplicate. A typical scheme is shown below for a weak estrogen of interest as well as 17β-estradiol (E2) (positive control). Concentrations are expressed as final concentration (M) in the 2ml media.
4. Incubate 20hr at 37C.



Harvesting of cells:

1. Aspirate off media.
2. Rinse each well twice with 1ml cold PBS.
3. Place plates on incline and aspirate off all remaining PBS.
4. Add 100µl lysis buffer (see recipe) per well with freshly added protease inhibitor cocktail (to 1x) and dithiothreitol (DTT; for 2mM final concentration add 8µl of 1M DTT per well).
5. Tap plates firmly to distribute lysis buffer.
6. Freeze plates for at least 20min.
7. Thaw plates, tap firmly again, and place on incline until cell debris sinks to the bottom and upper clear lysate can be removed.

Luciferase assay:

1. Add 10µl of cell lysate from each well to duplicate wells of an opaque 96-well plate. Because each treatment is represented by duplicate wells in the 6-well plate, there are now four wells per treatment in the 96-well plate.
2. Add 100µl Luciferase Reaction Buffer with 2mM freshly added DTT (add 0.2µl of 1M DTT per well) and 2mM freshly added ATP (add 2µl of 100mM ATP per well).

3. Set Luminoskan 96-well luminometer to inject 25µl of 0.5x luciferin (Molecular Probes; diluted in Luciferase Reaction Buffer) and read for 10sec.

β-Galactosidase assay:

1. Add 10µl of cell lysate from each well to wells of a transparent 96-well plate.
2. Add 100µl of β-galactosidase reaction buffer with 2.7µl β-mercaptoethanol per well freshly added.
3. Add 25µl 4mg/ml o-nitrophenyl-B-D-galactopyranoside (ONPG; Sigma).
4. Incubate until a pale yellow color appears (0.5-2hrs).
5. Measure absorbance at 420nm.

Calculations:

Luciferase activity for each well (units/µl/hr) =

$$\frac{(\text{luc reading} - \text{blank luc reading})}{(\beta\text{-gal reading} - \text{blank } \beta\text{-gal reading})} \; \frac{1}{(\mu\text{l of lysate used, i.e. } 10\mu\text{l})} \; \frac{1}{(\text{hrs before reading } \beta\text{-gal})}$$

Fold induction =

$$\frac{(\text{luc activity in well of interest})}{(\text{luc activity in vehicle well})}$$

Notes:
*calculations are averaged across the four readings for each treatment.
*typical fold induction for highest concentration of E2 relative to DMSO is ~20-fold

RECIPES:

TE:
1mM Tris (0.158g/l)
0.1mM EDTA (0.372g/l)
*dissolve in glass-distilled H_2O
*pH to 8.0
*sterilize with 0.22µ filter
*store at 4C

2x HBS:
280mM NaCl (16g/l)
10mM KCl (0.74g/l)
1.5mM $Na_2HPO_4 \cdot 2H_2O$ (0.27g/l)
12mM dextrose (2g/l)
50mM HEPES (10g/l)
*dissolve in glass-distilled H_2O
*pH to 7.05
*sterilize with 0.22µ filter
*store at 4C; long-term -20C

2M $CaCl_2$
2M $CaCl_2 \cdot 6H_2O$ (367.5g/l)
*dissolve in glass-distilled H_2O
*sterilize with 0.22µ filter
*store at -20C

5x Lysis Buffer:
125mM glycylglycine, pH 7.8 (25ml of 500mM/100ml)
20mM EGTA (4ml of 500mM/100ml)
50% glycerol (50ml of 100% glycerol/100ml)
5% Triton X100 (5ml of 100%/100ml)
0.75mM Spermine (0.75ml of 100mM/100ml)
300mM KCl (9.7ml of 3.1M/100ml)
75mM NaCl (1.5ml of 5M/100ml)

Luciferase Reaction Buffer:
25mM glycylglycine (25ml of 500mM/500ml)
15mM $MgSO_4$ (7.5ml of 1M/500ml)
4mM EGTA (4ml of 500mM/500ml)

β-Galactosidase Reaction Buffer:
60mM Na_2HPO_4 (30ml of 1M/500ml)
40mM NaH_2PO_4 (20ml of 1M/500ml)
10mM KCl (5ml of 1M/500ml)
1mM $MgSO_4$ (0.5ml of 1M/500ml)

100x Protease inhibitor cocktail:
1µg/µl Aprotinin
100µg/µl phenylmethylsulfonyl fluoride (PMSF)
others can be added as described in Molecular Cloning (Sambrook, Fritsch, and Maniatis)

Trypsin:
1. Prepare Solution A as follows:
   NaCl 4.2g
   Tris base 1.6g
   KCl 0.2g
   Na_2HPO_4 0.52g
   1N NaCl 7.5ml
2. Mix and allow to stand at 4C overnight.
3. Adjust pH to 7.4 and adjust volume to 500ml with water.

4. Mix together 450ml Solution A with 0.25g trypsin and 1.25ml of 0.2M EDTA (pH 7.5) and adjust volume to 500ml with water.
5. Sterilize through a 0.22µ filter.

Dextran-Coated Charcoal-treated Fetal Bovine Serum (FBS-DCC):

A. Preparation of DCC – make fresh every time.

1. Suspend 3.12g of activated charcoal (BDH – decolorizing powder activated, 33032 4E) into 50ml of 10mM tris (pH 7.4) in a 50ml disposable centrifuge tube.
2. Centrifuge 10min at 180g. Remove Tris and repeat two more times with fresh Tris.
3. Resuspend pellets in 50ml of 10mM Tris and transfer to a sterile 100ml bottle.
4. SLOWLY, while stirring, add 0.31g dextran T70 (Pharmacia) and continue to stir for 20min.

B. FBS stripping – 500ml.

1. Add 100 IU sulfatase (ICN) to a 500ml bottle of FBS (Intergen).
2. Incubate 2hrs at 37C with magnetic stirring.
3. Add 10ml fresh DCC.
4. Incubate overnight at 4C with magnetic stirring.
5. Centrifuge at 4200g for 10min. Pour supernatant into a fresh bottle.
6. Add 10ml fresh DCC.
7. Incubate with magnetic stirring at 56C for 45min.
8. Cool with magnetic stirring to 4C in ice bath (~30min).
9. Repeat steps 5-8 two more times.
10. Centrifuge a final time at 4200g for 10min. Pour supernatant into a fresh 500ml bottle.
11. Filter the stripped serum into a sterile 500ml bottle using a 0.22µ filter (e.g. Gelman VacuCap90). Do not overload the filter – may need to change filter several times.
12. Aliquot 25ml of filtered serum into 50ml sterile tubes and store at -20C.

Appendix B3

Protocol for the Development of new reporter gene assay systems for screening Endocrine Disrupters

(Provided by Drs. Mitsuru Iida and Teruhisa Kato, Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan)

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Development of new reporter gene assay systems for screening Endocrine Disrupters

EcoScreen assay ™ (high throughput transfection assay)

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INTRODUCTION

There is a great need for effective in vivo screening methods for detecting (anti)estrogenic and (anti)androgenic chemicals. We have developed rapid and sensitive reporter gene assays for detection of the chemicals that have agonist and antagonist activity against the estrogen, androgen, and thyroid hormone receptors. We believe that these methods have the potential to become powerful tools for identifying endocrine disrupters.

MATERIALS and METHODS

Chemicals

17-beta-estradiol, 5-alpha-dehydroxy testosterone, T3, T4, dimethylsulfoxide (DMSO) and rat S-9/Cofactor A Set were from Wako (Osaka, Japan). MTT was from Dojin (Osaka Japan) ALAMABLUE™ from Serotec (Oxford UK). The test solutions were prepared from stock solutions in DMSO and then 10 times serial dilutions were made with DMSO and finally diluted 100 times in the culture media with no supplement (the final DMSO concentration in the media was 1.0%). The test samples were adjusted to the concentrations ranging from 10^{-11} M to 10^{-5} M.

Samples for Estrogen and Androgen reporter assay (agonist activity detection)

The estrogen receptor agonist assay and androgen receptor agonist assay were carried out on 61 chemical compounds (See Appendix1 CHEMICAL LIST) designated by the Japanese Ministry of Economy and Industry for studies on the feasibility of screening for endocrine disrupters. All measurements were done in quadruplicate. We repeated this assay 2 times, and the results were in very good agreement. The configuration of the samples on a 96 well plate is shown in Figure 1-a and 1-b. The wells in row H contained positive and negative controls. Results are shown in figures in the appendix. Samples were identified as HTS “No”.

Samples for Estrogen and Androgen receptor antagonist activity detection assay

The samples listed in Table 1a, and 1b were examined for activity as antagonists for ER and AR as described below.

Plasmids

For estrogen receptor reporter gene assay

pGL3ERE-7: an estrogen responsive reporter plasmid harboring the TATA box from herpes simplex virus thymidine kinase (tk) promoter (1) and four copies of estrogen response element (2), linked to the luciferase gene.

pcDNA ER-alpha: mammalian expression vector for estrogen receptor-alpha with Zeocin resistant gene.

For androgen receptor reporter gene assay

pIND ARE B10: contains the hygromycin resistant gene and 4 copies of the androgen response element: (AGTACG nnn TGTTCT) from the C3 gene (3), linked to the luciferase gene.

pZeoSV2AR: An expression plasmid with the androgen receptor driven by the SV40 promoter, and the Zeocin resistance gene.

For thyroid hormone receptor reporter gene assay

PINDTRE: contains 4 copies of the thyroid response element TRE pal: GGTCATGACC)(5) linked to the luciferase gene.

pZeoSV2TR-beta: expression plasmid containing the thyroid hormone receptor-beta driven by SV40 promoter and the Zeocin resistance gene

For cell viability/non specific inhibition assay

pGL3 control: luciferase expression vector driven by CMV promoter

pcDNA-EGFP: mammalian expression vector containing the green fluorescence protein cDNA.

Estrogen Receptor Agonist Activity detection assay.

1st day: Chinese Hamster Ovary cells (CHO- K1) were maintained in DMEM/Fl2 supplemented with 100 U/ml penicillin, 100ug/ml streptomycin, and 10% fetal bovine serum. The cells were trypsinized and suspended at 1x 10^5/ml. They were seeded with 84 ul of culture medium in 96 well microtiter plates (Nunclon™ #137101, NalgeNunc Denmark) in DMEM/F12 containing 5% charcoal-treated fetal bovine serum (Hyclone, Logan, UT) and incubated for 24 hr at 37 °C in a humidified atmosphere of 5% CO_2.

2nd day:

Preparation of Plasmid cocktail: For one 96 well plate assay, 6 ug of pGL3ERE-7 and 60 ng of pcDNA ER-alpha (100:1) was added to a 1.5 ml eppendorf tube. The total volume of DNA solution was kept below 50 ul.

Preparation of transfection mix per one 96 well plate: 18 ug of nonliposamal transfection regent Fugene™ (Roche Diagnostic Corp. IN USA) were added to 660 ul of DMEM/F12 (with no supplement) in a small sterile tube. Then the plasmid cocktail (see above) was added to the tube and incubated for 25 min at room temperature.

Transfection: 6 ul of the transfection mix were added into each well of the seeded 96 well plate by multi channel pipet, and then incubated 3 hr. After incubation, 10 ul of

each chemical diluted with the culture media (see Chemicals) were added, and the cells incubated for 16-24 hr.
3rd day: Followed incubation, 100 ul of the luciferase substrate with cell lysis reagent Steady-GloTM (Promega) was added to all assay wells. After shaking at room temperature for 5 min. the luminescence was measured in an ARVO multi-label counter (Perkin-Elmer).
(see Appendix 2 for the scheme of the high throughput transfection assay)

Note 1
We found that the most stable and reproducible data were obtained when Nunclon™ plates (NalgeNunc, Denmark) were used. Plates from other manufacturers often gave high backgrounds, perhaps because ingredients in the plastic were stimulatory.

Note 2
Another source of variability is in the accuracy of dispensing the transfection mixture into the wells, if the distribution is done manually. The use of devices for automated delivery can reduce this source of error. However, if this is not feasible we have found that another seeding and transfection protocol is useful.

Alternative method for transfection
1st day: CHO-K1 cells were trypsinized and prepared at a density of 1x 10^5/ml. 11ml of cell suspension were placed in a sterile 50 ml conical tube (for one plate). The transfection mix was added (see original protocol) to the 50 ml conical tube, mixed gently, and incubated for 15 minutes. Then each well was seeded with 90 ul of cell suspension and incubated for 16-24 hr.
2nd day: 10 ul of test sample (see original protocol) were added to the wells and incubated for 16-24 hr.
3rd day: Same as original protocol.
(see Appendix 3 for the scheme of the alternative transfection method)
This method is easy to perform. Although the signal intensity may decrease because the transfection efficiency decreases, there is still sufficient intensity for measurement.

Estrogen Receptor Antagonist Activity detection assay

The protocol for antagonist activity detection assay is the same as agonist detection assay except that cell viability is evaluated by measuring the fluorescence of EGFP prior to the luminescence measurement. Only the differences between the protocols are described here.

1. Plasmid cocktail: 6 ug of pGL3ERE-7 and 60 ng of pcDNA ER-alpha + 480 ng of pcDNA-EGFP.
2. The test solutions were prepared using “Spiked Media” that contains 5x10^{-11} M of 17-beta-estradiol.
3. After the final incubation period of 24 hr, green fluorescence was measured (excitation: 485 nm, emission: 535 nm) prior to the luminescence measurement by the ARVO multi-label counter (Berthold).

Androgen Receptor and Thyroid Hormone Receptor Agonist / Antagonist Activity detection assay

They are the same as described above except for the use of different plasmids for expression of each receptor and the luciferase-reporter containing the corresponding hormone response element.

For Androgen Agonist detection assay

Plasmid cocktail: 6 ug of pIND ARE B10 and 240 ng of pZeoSV2AR.
5-alpha dehydroxy-testosterone (DHT) was used as a positive control.

For Androgen Antagonist detection assay

Plasmid cocktail: 6 ug of pIND ARE B10 and 240 ng of pZeoSV2AR + 480 ng of pcDNA-EGFP. The test solutions were prepared using "Spiked Media" that contains $5x10^{-9}$ M of 5-alpha-DHT

For Thyroid Hormone Receptor Agonist detection assay

Plasmid cocktail: 6 ug of pINDTRE and 120 ng of pZeoSV2TR-beta.
T3 was used as a positive control.

For Thyroid Hormone Receptor Agonist detection assay

Plasmid cocktail: 6 ug of pIND TRE and 120 ng of pZeoSV2TR-beta and 480 ng of pcDNA-EGFP
The test solutions were prepared using "Spiked Media" which contains $5x10^{-8}$ M of T3.

Cell proliferation assay for evaluation of cell viability in antagonist activity assay

CHO-K1 cells were transfected with 6 ug of pGL3 control plasmid and 480 ng of pcDNA-EGFP by the same method as the above-mentioned protocol with Fugene™, and cultured with different concentrations of DMSO, from 0% to 10%, for 24 hr. DMSO inhibits cell growth, and thus serves as a model for a non specific expression and growth inhibitor. Then the luciferase activity and EGFP fluorescence were measured. The MTT assay and ALAMARBLUE™ cell proliferation assay were also done in order to determine the reliability of the GFP assay as an indicator of nonspecific inhibition/cytotoxicity in the actual antagonist detection assays.

Data Analysis

Definition of PC50 (50% of Positive Reaction)

Chemicals that can be used to determine an EC50 (half maximal activity of a particular compound) are limited to a small number that have a similar activity/toxicity profile as E2. This is because the activity curves of many compounds do not reach a plateau before the maximum tolerated dose is reached. In order to compare the activity of chemicals whose activity does not reach a plateau at the maximum tolerated dose we have defined the PC50 as the concentration of compound that corresponds to ½ the value of the transcriptional activity of the positive control (10^{-9} M of 17-beta-estradiol). Thus the PC50 can be used to rank compounds when true half maximal values cannot be determined because of toxicity (Refer to fig. 2). This PC50 concept is based on the guideline of the Japanese Ministry of Economy and Industry. The use of the PC50 removes the requirement for a standard curve at every measurement. (Only a solvent control and positive control at plateau level are needed). We have found that there is

very little variance in the PC50 value from experiment to experiment, even with discernable variation in luciferase activity due to differences in culture condition and transfection efficiency.

To determine the PC 50, one concentration of the standard at the maximal activity level was included in each assay as a positive control (for ER assay: 10^{-9} M of 17-beta estradiol; for AR assay: 10^{-8} M of 5alpa-DHT). In each assay the reaction curve of the sample (ranging from 10^{-12} M to 10^{-6} M), and the ½ maximal point was determined by analysis of the data by a Cubic Spline Curve Fitting Method using soft ware designed by us.

RESULTS AND DISCUSSION

Estrogen receptor agonist activity

Table 2 shows the rank order of compounds that have ER agonist activity on the basis of the PC50 determination. DDT (HST0099) and DEE (HST0100) have detectable ER agonist activity, but do not reach the PC50 level (see Appendix 4: results of ER agonist assay). Assessment of compounds with weak activity requires the use of comparison standards adjusted to lower activity such as PC40 (40% of maximal positive reaction) or PC30 (30% of maximal positive reaction).

Androgen receptor agonist activity

Table 3 shows the rank of the compounds that have AR agonist activity on the basis of the PC50. Most of the listed compounds were natural ligands or synthetic steroid hormones. The results are shown by the graph in appendix 3. Progesterone (HST0008) and Aldosterone (HST0009) showed only slight reaction, and RU486 (HST0087) and Cortisol (HST0099) were about 40% of the reaction of positive control at the highest concentration (10^{-6} M).

Thyroid hormone receptor agonist activity

Four sub types of the thyroid hormone receptor (TR) are known: alpha1, alpha2, alpha3, and beta. We have performed assays with reporter plasmids for TR-alpha1and beta. Figure 3 shows the result of agonist assay of TR-beta receptor. There was about a 20 fold induction relative to the solvent control (0.1%DMSO) with 100nM T3, with a detection limit of 500 pM and PC50 of 2 nM. We have not carried out large-scale screening for TR receptor. However, after testing about100 compounds, we found that only T3 and T4 had clear agonist activity.

Determination of non-specific inhibition/cell toxicity in the antagonist activity assay

In the antagonist activity assay a constant amount of the standard ligand was added to the test sample containing the unknown. The antagonist activity was observed as a decline in the luciferase activity. It is essential to distinguish a decline in luciferase activity due to true receptor antagonism from the non-specific inhibition of expression or cell toxicity that some compounds display.

Fig.4 shows how the reporter activity was affected by the nonspecific inhibitory activity of a test sample, using DMSO as an example. CHO-K1 cells were transfected with pcDNA-EGFP (green fluorescence protein expression plasmid) and pGL3 control (luciferase expression plasmid), and were cultured in various concentrations of DMSO. Expression of the markers in both plasmids is constitutive. In 4% DMSO the activity of EGFP and Luciferase fell to 5% or less of control. However, the ALAMABLUE™ assay and MTT assay reported 56% and 88%, respectively, of the 0% of DMSO control. This experiment showed that expression of genes on the plasmids was more sensitive to DMSO than the other assays. The MTT assay, which measures the reduction activity of the intracellular dehydrogenases, is widely used as an index of the cell proliferation or cell number. The ALAMABLUE™ assay is a simple method suitable for measuring large number of samples, and is said to be well correlated with the MTT assay. The ALAMABLUE™ method measures change of the reduction/oxidation state of the culture environment as a result of cell proliferation. However, our results indicate that these assays are not reliable indicators of nonspecific inhibition of plasmid gene expression. The pattern of decline in expression of EGFP was in good agreement with that of luciferase. Consequently we monitor nonspecific inhibitory/cell toxicity effects of the samples by measuring the expression of EGFP in the receptor activity assays. This is straightforward, and can be performed on living cells in a 96 well plate format. Usually this measurement is taken just before measuring the activity of luciferase. The advantage of this strategy is that both EGFP and luciferase assays can be performed on the same cells.

Estrogen receptor antagonist activity

Fig.5a shows the result of the estrogen receptor antagonist assay for tamoxifen (CAS No.10540-29-1: anti-cancer drug). The GFP fluorescence is shown by the green line, and the luciferase activity by the yellow line. At the concentration of 10^{-7} M, the luciferase activity was18% of the control, while the GFP activity was about 100%. This shows that tamoxifen is an antagonist of the estrogen receptor. The antagonist activity of 4-hydroxy tamoxifen is about 100 times stronger than that of the tamoxifen (Fig.5b). With triphenyltin chloride (fig.5c), at 10^{-6} M, GFP showed 93% of activity, while the luciferase activity was about 75% of control. In another set of experiments we compared the antagonistic activity of a styrene dimer with, or without, metabolic activation by incubation with a rat liver S9 preparation. We found that without S9 treatment 1-Methly-1-phenylindan (styrene dimer, 10^{-6} M) showed no receptor antagonistic activity, while with S9 treatment the activity of GFP (red triangle) was 91% of control while the luciferase activity (blue triangle) fell to 60%. Although this requires additional study, metabolites of this compound may have weak antagonist activity against the estrogen receptor, while the parent compound does not.

androgen receptor agonist activity

Fig 6a-h shows the results of the androgen receptor antagonist detection assay. Cyproterone acetate (fig.6a) showed the strongest antagonist activity to the androgen receptor of all the compounds we have tested. Consequently we use cyprotenone acetate as a positive control in every measurement. Two pesticides, hydramethylnone (fig.6b) and tralomethrin (fig.6d), were judged to have no true antagonist activity, because the

decline in the luciferase activity was matched by the decline in GFP activity. Two pesticides (CNP: fig.6c, fenitrotion: fig.6e) were clear antagonists. At 10^{-6} M GFP expression was unaffected while luciferase activity was reduced to 23% (CNP) and 13% (fenitrotion). Weaker antagonistic activity was shown by prothiofos (fig.6f) and vinclozolin (fig.6g).

Spironolactone (fig.6h) gave a biphasic activity curve. At low concentrations (10^{-8}–10^{-6} M) in the presence of testosterone it was an antagonist, while at higher concentrations the antagonistic activity was reversed. In the absence of testosterone it was an agonist at high concentrations (10^{-5}–10^{-6} M). These results suggest that for some compounds the definition of antagonist and agonist will have to be qualified by an indication of concentration and the presence of other ligands.

CONCLUSION

Other reporter cell lines that constitutively express steroid receptors, in some cases several receptors, have been developed. For example, T47D expresses ER-alpha and ER-beta, Androgen, Progesterone, and Retinoic acid receptors. There is a Hela cell derivative that expresses the glucocorticoid receptor, while MCF-7 naturally expresses ER-alpha and ER-beta receptors. These lines will report the activity of compounds that stimulate any of the receptors and cannot distinguish which receptor(s) have been stimulated. The strategy we have employed measures the signal from only the receptor introduced by transfection since the CHO cells do not express any endogenous steroid receptor.

Our method can be considered a “ high throughput transfection assay ”. Generally these methods are thought to suffer from variability and lack of reproducibility, due in part to toxicity of the transfection reagent, and uncontrollable variation in cell culture conditions. However we have found that recently developed transfection reagents solve many of these problems. FuGene™ is in one of these reagents. A reporter gene assay using FuGene™ has been reported previously by Vingaard (4). This reagent does not show any toxicity to the cells and if methods for accurate delivery of reagents are established there is little intra-assay variation in measurement. We have found that the average intra-assay coefficient of variation was only 5.9% (CV5.9%) in assays of over a hundred compounds. Our method is simple and affords a significant reduction of lab work and produces reliable data. Actually our method does not have any medium exchange and plate washing step after seeding a cell on 96 well plate until measuring luminescence. If the measurement is carried out on the concentration of 4 doses in duplicate the cost per sample (except for personnel expenses) will be $10 or less. In conclusion, our method is suitable for pre-screening a large number of environmental chemicals and should identify compounds that need further testing.

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Appendix B4

Protocol for the Development of stably transfected cell lines to screen Endocrine Disrupters

(Provided by Drs. Mitsuru Iida and Teruhisa Kato, Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan)

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Development of stably transfected cell lines to screen Endocrine Disrupters

ER-EcoScreen assay™ and AR-EcoScreen assay™ (Stable CHO clones containing luciferase based reporter gene and expressing hormone receptors)

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INTRODUCTION

We have developed genetically engineered stable transfected cell lines, expressing hormone receptor and luciferase based reporter genes, for screening compounds and compound mixtures for endocrine disrupter activity. We have named the lines "**ER-EcoScreen™** " (expressing estrogen receptor) and "**AR-EcoScreen™** " (expressing androgen receptor). To establish these cell lines we introduced the plasmids used in our transient transfection Eco-Screen Assay™ systems. We have demonstrated that the cells have the same reactivity to the samples tested in the Eco-Screen Assay™ system. We also have confirmed that these cell lines do not lose reporter activity during continuous cell passage.

METHODS

Stable transfection of Hormone Receptor and Reporter Gene in CHO-K1 cell

About 16 hr prior to transfection, CHO-K1 cell were seeded at 50% confluence in a 6-well plate in 2 ml culture medium per well. Transfections were carried out with Fugene™ according to the Instruction Manual. For the estrogen receptor (ER) reporter assay, 12 ug of pINDERE-15 (containing the luciferase gene, under the control of the minimal heat shock promoter with 4 copies of the estrogen response element, as well as the hygromycin resistant gene) and 120 ng of pcDNA ER-alpha (estrogen receptor expression plasmid). For the androgen receptor (AR) reporter assay, 12 ug of pIND ARE B10 (4 copies of the androgen response element linked to luciferase, and the hygromycin resistance gene) and 480 ng of pZeoSV2AR (androgen receptor expression plasmid) were transfected per well. After 24 hr the cells were trypsinized and the cells from each well plated in two 100-mm petri dishes. The culture medium was replaced every three days with medium containing 200 ug/ml of Zeocin and 200 ug/ml of Hygromycin until colonies were large enough to isolate (about 10 days). Luciferase-positive clones were isolated using a photon detecting CCD camera (Night OWL, Perkin-Elmer). Briefly, clones were exposed to 0.2 nM luciferin and 1 nM E2 for the ER assay, 10 nM testosterone for the AR assay, for 24 hr, and then introduced into the CCD camera. Luminescence intensity was monitored for 10 min per dish and the

luminescence image from cell was superimposed on to the light field image of the cell clones in the dish. Positive clones were isolated using cloning rings and further cultured in 24 well plates. After growth each clone was trypsinized and seeded into 2 wells in two 96 well plate (Nunclon™ NalgeNunc Denmark) and further cultured. After 24 hr culture, cells in one plate were exposed to 0.1% DMSO as a control, while the cells in the other plate were incubated with 1nM of E2 for ER assay and 1nM of 5-alpha-dehydrotestosterone for AR assay, respectively. Followed 24 hr culture, 100 ul of the luciferase substrate with cell lysis reagent Steady-Glo ™ (Promega) were added to all assay wells. After shaking at room temperature for 5 min the chemiluminescence was measured in the ARVO multi-label counter (Perkin-Elmer). The most responsive clone was selected.

Procedure for ER/AR-Eco screen assay™

1st day: The most responsive CHO-K1 stable clone was maintained in DMEM/Fl2 supplemented with 100 U/ml penicillin, 100 ug/ml streptomycin, and 10% fetal bovine serum. The cell were trypsinized and prepared at a density of $1x10^5$/ml, and then seeded with 90 ul of culture medium in 96 well microtiter plates (Nunclon™ #137101, NalgeNunc Denmark) in DMEM/F12 containing 5% charcoal-treated fetal bovine serum (Hyclone, Logan, UT) and incubated for 24 hr at 37 °C in a humidified atmosphere of 5% CO_2 / air.

2nd day: After 24 hr culture 10 ul of sample solution from serial dilutions of each chemical with the culture media (see Chemicals on protocol 1) were added to the plates and cultured for 16-24 hr.

3rd day: Followed 24 hr culture, 100 ul of the luciferase substrate with cell lysis reagent Steady-Glo™ (Promega) were added to all assay wells. After shaking at room temperature for 5 min the chemiluminescence was measured by ARVO multi-label counter (Perkin-Elmer).

Chemicals for Estrogen and Androgen reporter assay (agonist activity detection)

The estrogen receptor agonist assay and androgen receptor agonist assay were carried out with 12 chemicals for ER-EcoScreen™ and AR-EcoScreen™**,** respectively (Table 1 and 2).

Data Analysis

We used the criteria of PC50 for data analysis. Refer to the “Definition of PC50” on a report 1 ”Development of new reporter gene assay systems for screening Endocrine Disrupters.” The data were analyzed with software by applying Cubic Spline Curve Fitting Method. EC50 is also shown for reference.

RESULTS

Clone stability for ER-Eco Screen™

The cloned line (ER-Eco Screen™) that was stably transfected with pINDERE-15 and pcDNA ER-alpha showed stable expression over at least 15 passages during more than two months of culture (fig.1-a).

In over 10 assays, this clone reported about 3.5 fold induction with 100 pM E2 compared to solvent treatment (0.1%DMSO), with a detection limit of 0.5 pM. The PC50 was 9.2 pM.

Clone stability for AR-Eco Screen™

Our cloned AR-Eco Screen™ was stably transfected with pIND ARE B10 and pZeoSV2AR. It was responsive to DHT for 30 passages over three months culture (fig.1-b), as observed in over 10 assays. This clone showed about a 5 fold induction with 1nM DHT compared to solvent (0.1%DMSO), with a detection limit of 15 pM. The PC50 was 153 pM.

Estrogen receptor agonist activity

Table 1 shows the rank of the compounds that had ER agonist activity on the basis of the PC50. Although there were slight differences, the ranking on the basis of PC50 was almost the same as that of the high throughput transfection assay. (Refer to Table 1 on the report of "high throughput transfection assay") The reaction curves for all measurements are shown in appendix 1. Although DDT (HST0099) and DEE (HST0100) had detectable ER agonist activity, they did not reach the PC50 (see appendix). As noted before, with weakly active compounds, standards such as PC40 (40% of positive reaction) or PC30 (30% of positive reaction) are more useful for ranking purposes.

Androgen receptor agonist activity

Table 2 shows the rank of the compounds, which have AR agonist activity, on the basis of the PC50. The reaction curves for all measurements are shown in appendix 2. Most of listed compounds that showed high agonistic activity were natural ligands or synthetic steroid hormones. Progesterone (HST0008) and Aldosterone (HST0009) showed only slight activity. RU486 (HST0087) and Cortisol (HST0099) at the highest concentration (10^{-6} M) examined were about 40% of the reaction of the positive control. The results were almost same as that of high throughput transfection assay. (Refer to Table 2 on the report of "high throughput transfection assay").

DISCUSSION

In our presentation of the high throughput assay we discussed the problem of ranking weakly active compounds, those whose reaction curves failed to reach one half of the plateau level of the positive control, and for which a PC50 could not be calculated. In some cases, although a PC50 could not be determined, the reaction curves did plateau (Estrone (THS00022), RU486 (HTS00087) and Cortisol (HTS00088), in the androgen agonist assay), and so an EC50 could be calculated (see table 2 and HTS00022, HTS00087, HTS00088 on Appendix 7). Comparing chemicals with weak and strong activity on the basis of EC50 determinations can be controversial and cause some with genuine activity to be disregarded. The PC50 ranking is a practical approach and weaker compounds can be qualified in terms of PC40 (40% of positive reaction) or PC30 (30% of positive reaction). The results presented above show that

both cell lines can distinguish compounds with strong activity and with weak activity, and the results can be used to rank the compounds.

As pointed out in our report on high throughput screening, others have developed cell lines with stable transfected reporter genes. These include MCF-7 (4), Hela (5), T47D (6), and PC-3 (7) cells. These lines all express multiple steroid receptors. For example, T47D cells express ER-alpha and ER-beta, androgen, progesterone and retinoic acid receptors. Therefore, in the assays, cross- reaction may be observed, and it is impossible to distinguish whether ER-alpha or ER-beta has bound ligand. PC-3 cells actively metabolize steroids, and so natural ligands like testosterone and 5alpha-DHT cannot be used as standards. In contrast the CHO-K1 cells do not metabolize steroid hormones and do not express endogenous steroid receptors. Thus it is possible to measure the signal from only the transfected receptor.

CONCLUSION

This method is suitable for high throughput screening applications, and generates reliable data.

POSTSCRIPT

We continue to improve our system. Recently we have derived clones that give stronger signals on receptor activation, and thus are more sensitive. We are now preparing cell lines that express both EGFP and the reporter system simultaneously, and our preliminary results are promising. These will be developed for receptor antagonist activity assays, similar to those described in the transfection assay system.

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Appendix B5

Technical Perspective on the U.S.E.P.A. Endocrine Disruptor Screening Program: In Vitro EDSTAC Guideline Protocols

(Provided by Dr. Grantley Charles, Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, MI, USA, and Dr. William Kelce, Pharmacia Corporation, Kalamazoo, MI, USA)

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Technical Perspective on the U.S. EPA Endocrine Disruptor Screening Program:

In Vitro EDSTAC Guideline Protocols[1]

I. Introduction

The Food Quality Protection Act of 1996, amending the Federal Food, Drug and Cosmetic Act, directed the Environmental Protection Agency (EPA) to develop a screening program to evaluate whether or not certain chemical agents could potentially have hormone-like effects in humans. The Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) convened by the EPA recommended a tiered testing approach for the evaluation of endocrine, androgen and thyroid related effects of commercial chemicals and environmental contaminants (EDSTAC, 1998).

Under this testing paradigm, Tier I screening would identify chemicals with a potential to affect the estrogen, androgen and thyroid systems. The recommendations of the EDSTAC for a Tier I screening battery encompassed the utilization of *in vitro* test system methodologies that recognize known mechanisms by which chemicals can interact directly with the estrogen, androgen and thyroid hormone systems. These *in vitro* assays included evaluations of direct binding to the hormone receptors as well the ability of test compounds to activate marker response genes (reporters), linked to hormone responsive genetic elements. The Tier I assays are intended for use in rapid initial screening and prioritization of chemicals for further definitive *in vivo* Tier II testing to determine any potential adverse effects of an endocrine-active substance.

Tier I *in vitro* assays are used as screening tools to provide mechanistic data. These data should not be used as the sole element in a risk assessment regulatory context for test compounds. The *in vitro* screening assays are intended to be used in a hierarchical system which includes, as appropriate, *in vivo* Tier I screening assays and *in vivo* Tier II tests. In this hierarchical system a negative Tier II outcome would supercede a positive Tier I finding (EPA, 2000).

There are limitations inherent in the recommended *in vitro* assays that restrict their effectiveness as large scale, precise, valid, screening tools (Holmes *et al.*, 1998; Zacharewski, 1998). These include but are not limited to:

- Inability to distinguish agonists from antagonists (receptor binding)
- Issues of limited metabolic capacity and bioaccumulation
- Limited/variable chemical uptake

[1] This technical perspective was prepared by experienced scientists engaged in *in vitro* and *in vivo* toxicological research and testing of industrial chemicals/ pesticides/pharmaceuticals. The primary authors of this commentary are listed under acknowledgements.

- Dependence on specific receptor or response element interactions not mimicked *in vivo*
- Lack of 'gold standard' protocols/methodologies for evaluation of assay results across laboratories
- Issues of proprietary and/or restricted use under US patent law regarding the use of human cDNA sequences coding for human nuclear hormone receptors (and/or simultaneous co-transfection of receptor and reporter constructs; cis-trans technology) for use in reporter gene transactivation assays

These limitations need to be addressed in order to maximize the potential use of these assays/methodologies in a properly functional, tiered, screening paradigm required for the assessment of adverse chemical effects on the endocrine system. This paper seeks to aid in moving forward the process of producing sensitive, specific, accurate and properly validated Tier I *in vitro* methods that could be used as screening assays for hormonal activity.

II. Major Elements To Be Considered for Standardization and Validation of In Vitro Assays

The following factors need to be taken into consideration in developing, validating and implementing *in vitro* assays for hormonal activity:

- There are at present several different methodologies for the performance of estrogen and androgen receptor binding (Nikov *et al.,* 2000; Blair *et al.,* 2000; Nagel *et al.,* 1997) and reporter gene transactivation assays (Pons *et al.,* 1990; Zacharewski *et al.,*1994; Kelce *et al*., 1995; Gaido *et al.,* 1997; Maness *et al.,* 1998; Vinggaard *et al.,* 1999). To date, the inter-laboratory variability, sensitivity, reproducibility and precision of these techniques have not been sufficiently evaluated. Furthermore, alterations in specific assay parameters can also lead to significant variability (Beresford *et al.,* 2000; Charles *et al.,* 2000). A single methodology therefore needs to be properly standardized and validated as the 'gold standard' by which other alternative protocols can be reliably compared.

- This gold standard *in vitro* protocol/methodology should be validated under an Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) type process in which several laboratories utilize identical protocols to assess the robustness of the assay in terms of reproducibility and accuracy. An agreed upon set of reference chemicals should be used to assist in the validation especially with regard to specificity and sensitivity.

- *In vitro* assays performed as part of the Tier I screening methodology should be performed in compliance with Good Laboratory Practice (GLP) provisions of the USEPA, OECD and/or MAFF so as to ensure the quality of the data derived from the studies. This includes the proper characterization of the test material for potential purity and/or contamination prior to assay utilization.

- A definite set of pass-fail criteria should be elaborated for each *in vitro* test system/methodology so as to minimize the potential confusion that may result from individual laboratory determinations. These would include criteria such as acceptable coefficients of variation (CVs), techniques for assessing cytotoxicity and definition of acceptable levels of cytotoxicity, required numbers of replicate data points per experiment, as well as cutoffs for designating a positive/negative response relative to defined controls.

- In light of the desire to minimize the number of animals that will be used in the implementation of any new toxicological testing procedures, the utilization of methodologies which make limited use of animals (e.g. recombinant receptor proteins for binding assays) should be promoted.

The following discussion provides technical perspectives and recommendations on the design, methodology, and evaluation criteria of nuclear hormone receptor binding assays and nuclear hormone transcriptional activation assays. In addition, the limitations of the testicular steroidogenisis assay are described. These perspectives and recommendations have been developed to promote technical discussions among the scientists engaged in the development, standardization and validation of *in vitro* methods for use as Tier I screening assays for hormonal activity.

III. Nuclear Hormone Transcriptional Activation Assays

III. A. Purpose & Background

The purpose of this procedure is to screen chemicals for the capacity to activate or inhibit ligand-induced transcription mediated by the mammalian estrogen and androgen nuclear receptors. The general premise is that nuclear hormone receptors bind ligand, which leads to alteration of their conformation, and subsequent binding to specific response element sequences on DNA and the initiation of transcription of the downstream gene. For convenience, the downstream gene codes for a protein (e.g., luciferase) that can be easily and accurately measured (i.e., a reporter gene) and therefore signals the potency of various ligands/chemicals to bind the receptor and either initiate or inhibit receptor-induced transcription of the reporter. Reporter gene assays then assess both agonist (test chemical alone) and antagonist (test chemical in the presence of stimulating ligand) activity.

In order to avoid potential US patent restrictions regarding the use of human cDNA sequence coding for human nuclear hormone receptors (and/or simultaneous co-transfection of receptor and reporter constructs; cis-trans technology), cell lines known to express endogenous human nuclear receptors are recommended. Cells expressing the human nuclear receptor of interest need only have the reporter gene introduced into them in order to be used for transcriptional activation assays.

Reporter genes can be transiently introduced into cells and used over the course of several days or stably integrated into the cells genomic DNA and used indefinitely, provided their responses to known ligands are stable and verified on a periodic basis. The response variability of transient expression systems is, however, an issue for routine use. Few stable cells lines for nuclear (estrogen and androgen) hormone receptor reporter gene assays are currently available, therefore the protocol recommended here uses accepted methods for transient reporter gene transfections.

III. B. General Assay Design

In brief, cells should be seeded into tissue culture plates, transiently transfected with the reporter gene, fed media containing treatment compounds with and without stimulating ligand. Following a defined treatment period, cell lysates are harvested and assessed for reporter (e.g. luciferase, β-galactosidase) activity. Concurrent with the reporter assay, an identically transfected and treated, satellite assays should be run and evaluated for cytotoxicity. For the screening of test chemicals, a dose-response assay is recommended in order to discriminate between highly potent ligands that may be cytotoxic at high concentrations from weak non-cytotoxic ligands that exhibit agonist activity at higher concentrations.

III. C. Recommended Design Features

The dose range should encompass the low pM range to the chemical solubility limit as the upper concentration to be evaluated. Alternatively, the upper limit should also be defined as that below which no cytotoxicity is observed.

Cells should be cultured aseptically in appropriate media using standard cell culture techniques. The optimal number of cells seeded into each dish or well should be determined empirically by each laboratory and is directly dependent on the transfection efficiency of the reporter gene. It is critical that seeding density is uniform, as alterations in cell number per well will introduce unnecessary variability in the assay.

The use of charcoal stripped serum is important to remove endogenous steroids from the serum which can activate transcription of the reporter gene and confound the experiment. A steroid free environment is especially important for estrogen receptor mediated transactivation experiments as many general laboratory procedures and supplies have been shown to artificially induce estrogen receptor mediated responses. In this context, laboratories should strive for an estrogen free environment.

Higher transfection efficiencies using lipofectin, FUGene or electroporation mean that fewer cells are necessary to induce an easily measured response and the assay can be completed using an efficient 96-well format. These transfection methods are recommended over more traditional calcium-phosphate precipitation and DEAE-Dextran that generally give much reduced transfection efficiencies.

Since the assays are generally performed in large multi-well formats, each plate should have its own positive and negative controls and should be considered a single experiment for data

analysis purposes. A concentration of the inhibitor control should be selected that reduces transcriptional activation by at least 90% in the presence of stimulatory ligand. Duplicate evaluations of each test chemical concentration should be assessed per experiment. Experiments should be replicated at least three times on different days.

17β-estradiol and 5α-dihydrotestosterone are recommended stimulatory ligands for the estrogen and androgen receptor assays, respectively. The concentration of stimulatory ligand used in test article antagonism studies should induce transcriptional activity to levels approximately 80-90% of maximum; use of sub0maximal levels insures that the receptor is not saturated with agonist ligand and incapable of responding to inhibitory compounds. ICI-182,780 and hydroxyflutamide (Wakeling *et al.,* 1991; Clark *et al.,* 1981; Kelce *et al*., 1995) are the respective recommended antagonism controls and should be used at concentrations that inhibit transcriptional activation by 90% or more. Other stimulatory and antagonist controls are acceptable provided that they are appropriately validated against the standard controls.

Control and test chemicals should be solubilized in ethanol or DMSO and added to the media in each well to a final concentration determined empirically as part of the initial standardization and validation effects for that cell line. Particular attention should be given to the solubility of test chemicals especially at the high doses. Any precipitate, discoloration, or persistent light refractive changes on the media surface should be noted and included in the final report indicating potential solubility problems. Other vehicles may be used provided appropriate determination of its effects on the cell line and reporter activity are properly standardized and validated.

III. D. Data Presentation and Pass-Fail Criteria

- The percent coefficient of variation (%CV) of replicate samples at each concentration of test or control chemical cannot exceed 20% in any assay. Data which exceeds the 20%CV at any concentration of test or control chemical within an assay will fail these criteria and all data for that concentration of test or control chemical for that particular assay must be excluded from the data analysis. All data failing these criteria should be so indicated in the data tables. The antagonist control must reduce transactivation by at least 90% within a 20%CV or the assay will be considered unacceptable.

- Data from transactivation experiments should be replicated at least three times each on different days. Data should be tabulated and graphed as reporter activity (relative light units) on the ordinate versus log dose of test chemical on the abscissa. For convenience, reporter data can be presented as %-control (%-maximal activity induced by stimulatory ligand) provided actual control values are clearly indicated.

- The EC_{50} (agonist experiments) is calculated as the concentration of test chemical that activates transcription by 50% relative to the maximal activity induced by stimulatory ligand.

The IC_{50} (antagonist experiments) is calculated as the concentration of test chemical that inhibits transcription by 50% relative to the maximal activity induced by stimulatory ligand. EC_{50} and IC_{50} values for each test chemical and the positive and antagonist controls, respectively, should be tabulated for each assay and the means together with a measure of the variability (e.g., standard deviation) from all assays clearly indicated.

- An efficacy of 25% of the positive control (or the negative control in the case of antagonist activity) should be considered a positive response for that test chemical in that assay.

III. E. Limitations

The following limitations of transcriptional activation studies should be recognized:

- Differences in sensitivity exist among clones of a given cell line (Villalobos *et al.,* 1995) in terms of their endocrine responses. Hence adequate characterization of cell lines are necessary and the testing methodology should address factors such as drift in responsiveness, sensitivity and specificity to minimize variability in response across laboratories.

- Test end points are dependent upon interactions with a given receptor structure or engineered response element. Therefore, results from any single gene transactivation system for a given chemical may vary significantly from that of another.

- Reproducibility of results will always be a potential concern, consequently, test systems should be widely available to enable confirmatory findings by other laboratories. It is therefore essential to perform an ICCVAM-type validation on a specific estrogen and androgen transactivation systems to act as gold standards to which other assay systems could be compared.

- It should be noted that transiently transfected cell lines exhibit some degree of variability across experiments in terms of their responses making stable cell lines a potentially more appealing alternative for validation purposes. In the event that new, stable cell lines are developed and are generally available, it is recommended that they be used with the caveat that they are properly validated in accordance with the ICCVAM principles already outlined. Their sensitivity, accuracy, precision and specificity should also be reviewed on a periodic basis to protect against genetic drift and cellular mutations that may compromise the integrity of the assay system.

IV. Acknowledgements

The primary authors of this technical perspective are Grantley Charles (Dow), William Kelce (Pharmacia) and Leonard Davis (DuPont).

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Appendix B6

Lyticase-based cell lysis protocol of β-Galactosidase assay for 96 well plates

(Provided by Dr. Rémy Le Guével of the Université de Rennes, Rennes, France)

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Lyticase-based cell lysis protocol of β-Galactosidase assay for 96 well plates

1 Media and solutions

Complete minimal (CM) dropout medium without leucine

Per 1 liter:
1.26 g dropout powder (a mix of essential amino acid, Sigma)
6.7 g yeast nitrogen base (YNB) without amino acids or ammonium sulfate (Difco)
5 g ammonium sulfate
10 g D-Glucose
Autoclave for 15 min.

Z buffer

Per 1 liter:
16.1 g Na_2HPO_4 7 H_2O (60 mM final)
5.5 g NaH_2PO_4 H_2O (40 mM final)
0.75 g KCl (10 mM final)
0.246 g $MgSO_4$ $7H_2O$ (1 mM final)
2.7 ml β-mercaptoethanol (50 mM final)
Adjust to pH 7.0. Do not autoclave

Lyticase 10X stock solution (1µg/µl)

Per 10 ml:
10 mg lyticase (Sigma)
1ml potassium phosphate buffer 1 M, pH 7.5
0.2 ml NaCl 5 M
5 ml glycerol,
Complete to 10 ml with H_2O
Store at –20°C.

ONPG substrate

Per 100 ml:
400 mg ONPG in potassium phosphate buffer 0.1 M pH 7.0
Filter sterilized and stored at –20 °C.

2 Cell growth

2.1 Growth on solid media

Yeast from glycerol stock were streaked on CM plates plus leucine and incubated at 30°C. Single colonies may be seen after 24 h but require 48 h before they can be picked for growth in liquid media.

2.2 Growth in liquid media

Four independent colonies were picked and inoculated in 4 Erlenmeyer flasks containing 5 to 10 ml CM medium plus leucine (or appropriate selective medium). The volume should not exceed 1/5 of the total flask volume. Yeast cells are incubated at 30°C in a shaking incubator at 300 rpm (the optimal speed depends on the orbital radius of the shaker) for 24 to 36 hours.

Note 1: It is important for all glassware to be detergent-free
Note 2: Other material than Erlenmeyer flask can be used (50 ml Falcon tube, for example).

2.3 Determination of cell density

The density of cells in liquid culture can be determined spectrophotometrically by measuring its optical density (OD) at 600 nm. For reliable measurements, cultures should be diluted such that the OD_{600} is <1 (generally 1/10 dilution in H_2O). In this range, 0.1 OD_{600} unit corresponds to ~$7x10^6$ cells/ml with our spectrophotometer. It is advisable to calibrate the spectrophotometer by graphing OD_{600} as a function of the cell density that has been determined by direct counting in a hemacytometer or titering by spreading on CM plates for viable colonies.

3 Assay for β-Galactosidase in 96-well plates

This protocol describes a rapid, quantitative assay of β-Galactosidase activity of recombinant yeasts for estrogen receptor (ER) in liquid culture. Yeast cells are lysed by hypo-osmotic shock after wall cell digestion by lyticase.

3.1 cell suspension seeding in conic bottom 96-well plate

After determination of the cell density, the culture is diluted in CM plus leucine to obtain 0.5-0.6 OD_{600} (see **2.3** for calibration of the spectrophotometer).For one 96 well culture plate 12-15 ml (in 15 ml Falcon tube) of cell suspension is required. Generally 3 plates from 3 independent colonies are used in parallel. 100 µl of yeast suspension are distributed in each well with multi-channels pipette (8 or 12 channel) excepted the first row (1 to 12 row or A to H column) which receives 200 µl of cell suspension. The first row containing 200 µl of yeast suspension is reserved for dilution of test compounds.

3.2 dilution of test compounds

Two µl of 100X test compounds (dissolved in ethanol or DMSO) are added in each well of the first row (200 µl of cell suspension) to obtain the first concentration (1/100 dilution of the stock solution). Mix gently 4 to 5 times the suspension with the multi-channels pipette. Dump 100 µl in the second row of the plate (1/2 dilution). Repeat this process for the other rows and eliminate the 100 µl in excess for the last row.

Note: Another procedure can be used. This consists of in making in separated tubes a series of dilution of 50 or 100 fold concentrated compounds in DMSO. One or two µl of each dilution are distributed in each well before cell suspension distribution. It is important in this process to use

DMSO as solvent to avoid evaporation of solutions in the plate during the distribution of compounds in each well. Ethanol is too volatile for this procedure.

3.3 Yeast cell incubation with test compounds

Plates are incubated for 4 hours at 30°C without shaking in an appropriated humidified incubator. Plates must be covered to avoid evaporation and they must not be directly exposed to the air flow of the incubator. This point is particularly important.

3.4 cell suspension recovering

After four hours of treatment, plates are centrifuged for 5min in an appropriated rotor for 96-well plates at 1500 rpm max (higher speed can break the plate). The supernatant is eliminated from each well using a vacuum pump.

At this step, cell pellets can be stored at –20 to –80°C for several days.

At this step, since the residual medium volume is low, the pellet can be gently resuspended using a vortex. This should be performed carefully to avoid cross-contamination between wells. This step is very important for an efficient action of lyticase in the next step.

4 Protocol of lyticase cell lysis

4.1 cell lysis

Dilute 10 time stock solution of lyticase (1µg/µl) in Z buffer solution. Fifty µl (5 µg) of lyticase in Z buffer is added in each well for 30 min at room temperature (20-22°C). Do not use vortex at this step. The temperature is very important for an optimal lysis during 30 min so that an incubator should be used if the room temperature was higher than 22°C.

After incubation with lyticase, 100 µl of 0.01% triton X100 solution in H_2O is added in each well and incubated for 15 min at room temperature. This hypo-osmotic shock is sufficient for a complete lysis of yeast cells but a freezing/thaw cycle at –80°C can be performed to obtain complete cell lysis if necessary (**see 4.2**).

Note: Do not freeze the extracts for a long time at this step to avoid apparition of protein aggregation. These protein aggregates will be pelleted with cell debris during the centrifugation in the next step and up to 90% of soluble proteins as well as β-Galactosidase activity can be lost after 3 days at –80°C.

4.2 β-Galactosidase assay and protein quantification

Plates are centrifuged in an appropriated rotor for 96-well plates at 1500 rpm max for 5 to 10 min. At this step, the pellets appear opalescent and more spread out than cell pellets; this indicates a good lysis of yeast cells. If the pellets appear very white and compact, this indicates a bad lysis of cells, In this case, perform freezing/thawing cycle after re-suspension of the pellets.

Aliquots of 100 µl of supernatants are transferred in flat bottom 96-well microtitration plates and 50 µl of ONPG substrate are added in each well. The enzymatic reaction is performed at 30°C for 1 to 2 hours for rtER and 30 mn to 1 hour for hER. Read the OD_{405} when the yellow color appears sufficient (0.8-1 OD_{405} for the maximal induction); stop the reaction by adding 50 µl Na_2CO_3 1M and read the plate after 5 minutes of equilibration.

During β-Galactosidase reaction, perform protein quantification. For this, 10 or 20 µl of soluble extract are added to 200 µl of Coomasie blue reagent. Add BSA standard: 0, 1, 2, 4, 6, 8 µg BSA per well and calculate the quantity of proteins in 150 µl of the total volume of lysis solution. The same correction is realized for OD_{405}.

Determine the β-Galactosidase units defined as OD_{405}/mg protein/minute of enzymatic reaction.

Note1: The accuracy of protein quantification is important for reproducibility of measurements of β-Galactosidase activity between independent experiments.

Note2: The definition of the β-Galactosidase is an arbitrary personal unit, not an official unit.

Appendix B7

High-Throughput System for Screening Estrogen-Like Chemicals

(Provided by Dr. George C. Clark,
Xenobiotic Detection Systems, Inc., Durham, NC, USA)

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High-Throughput System for Screening Estrogen-Like Chemicals Submitted by Xenobiotic Detection Systems, Inc.

1.0 Detailed procedures for conducting the test:

Mass culture of BG1Luc4E2 cell line: The cell line BG1Luc4E2 has remained stably transfected with the reporter plasmid for over 2 years. Early clones of the cells are stored in liquid nitrogen in 1 ml ampules. Mass culture of the cells are initiated by quickly thawing an ampule and suspending in 5 ml of RPMI 1640 containing 5% fetal calf serum (Hyclone) and 1% pen/strep solution (RPMI growth media) in a T25 culture flask. The cells are then incubated in a 5% CO2 incubator at 37C and allowed to grow to confluence (approximately 24 hrs). The adherent BG1Luc4E2 cells are rinsed with Phosphate Buffered Saline (PBS) and a 1% solution of Trypsin (Gibco) is applied to the cells in the flask and they are incubated in this solution for approximately 1 minute. Hit the side of flask sharply against heel of your palm to dislodge cells from the bottom of the flask and confirm release of the cells by visual examination with a inverted microscope. If not, allow to incubate for another 30 seconds and try again. If cells have been mostly dislodged, then add 5-6 milliliters PBS to the flask and wash the cells out of the flask. The cells are washed out of the flask with PBS and are transferred to a 50 ml centrifuge tube. RPMI 1640 Media containing 5% fetal calf serum is immediately added to the tube to inhibit further cellular digestion by residual Trypsin. The cells are pelleted by centrifugation in a desk top clinical centrifuge at 1000 RPM and resuspended in2 ml of RPMI growth media and repeatedly drawn through a pipet to break up clumps of cells. One ml of the pelleted cells is transferred inoculated into two T75 flask containing 10 ml of RPMI growth media and allowed to grow in the incubator at 37 C with 5% CO2 atmosphere until confluent. The cells from one T75 flask can be used to inoculate four T75 flasks by repeating the trypsin procedure for passage of cells described above.

Conditioning of BG1Luc4E2 cells for measuring estrogen dependent luciferase activity:
The BG1Luc4E2 cells must be grown in estrogen free conditions to allow measurement of estrogen dependent induction of luciferase activity [Rogers, 2000 #197]. The following procedure has been developed to condition the cells in estrogen free conditions and then allow plating of the cells in a 96 well plate format for HTPS analysis of estrogen dependent induction of luciferase activity. Two T75 flasks of cells grown in RPMI growth media that have reached confluence are removed from the incubator and the media is poured off of the cells. The cells are washed with 10 ml of PBS and then 10 ml of estrogen free media is added to the flask. Estrogen free media consists of Dulbeco's Minimal Essential Media supplemented with 5% fetal calf serum that has been stripped of estrogen by treatment with activated carbon and is free of phenol red pH indicator. The flasks of cells are returned to the incubator for 24 hours. At this time the cells are ready to be plated into 96 well plates. The two T75 flasks are treated with trypsin and cells washed with PBS and the cells are resuspended in approximately 15 ml of Estrogen free media. The cells are counted with a hemocytometer and adjusted to a concentration of 300,000 cells per ml in Estrogen free media. Two hundred microliters of media are dispensed into each well of a 96 well plate (60,000 cells per well). The plate is returned to the incubator of 24 hours to allow them to adhere and grow in the plate.

Dosing 96 well plates of BG1Luc4E2 cells with Estrogen and test compounds:
Dilutions of beta-estradiol and test compounds are prepared in DMSO. A standard solution of 10 ng/ml of beta-estradiol in DMSO is used to prepare dilutions of this standard. Four microliters

of DMSO is added to ten 13 mm glass tubes. To the first tube 4 microliters of the 10 ng/ml standard solution of beta-estradiol is added to the 4 microliters of DMSO in the tube. The tube is vortexed and four microliters transferred to the next tube in the series. This is repeated for each of the 10 tubes creating a two fold dilution series. To each tube 400 micoliters of Estrogen free media is added to the DMSO solution and the tube vortexed vigorously. Similar dilution series are produced for test compounds or extracts being analyzed for estrogenic activity by the BG1Luc4E2 cells.

The 96 well plates of cells are removed from the incubator and media removed from the adherent cells by inversion onto absorbent plastic backed paper. The cells are rinsed with 50 microliters of PBS and this also removed by inversion on absorbent plastic backed paper. Two hundred microliters of beta-estradiol solutions or test compound is then applied to the 96 well plates. The outside rows of the plate are not used for determinations since we have found that these wells are very sensitive to environmental conditions and do not provide reproducible quantitative readings for induction of luciferase activity with the BG1Luc4E2 cells. The dosed plates of BG1Luc4E2 are returned to the incubator and incubated for 24 hours to allow maximal induction of luciferase activity in the cells.

<u>Measurement of estrogen induced luciferase activity in BG1Luc4E2 cells:</u>
Luciferase that is produced in the BG1Luc4E2 cells in response to exposure to estrogen accumulates in the cytoplasm of the cells over the twenty-four hour incubation. To measure luciferase the cells must be lysed and substrates for measurement of luciferase enzyme activity added and results, light emission by the enzymatic activity measured in a luminometer. To accomplish this, the cells are removed from the incubator and media removed by inversion of the plate on absorbent plastic backed paper, and the plates tapped on the paper to remove residual media. The cells in the 96 well plates are washed with PBS and the cells examined with an inverted microscope to observe whether any observable toxicity or displacement of the lawn of cells grown on the bottom of the plates has occurred. The PBS is then removed by inversion of the plate on absorbent plastic backed paper and a reflective white plate tape (Packard) applied to the clear bottoms of the 96 well plates to increase the efficiency in measuring emitted light from the wells of the plate. A dilute detergent lysis reagent (Promega) is then added to the cells and the cells shaken in a vibrating mixer to aid in lysis of the cells. The cells are then placed in a Lucy 2 Luminometer (Anthos Analytical) which is a robotic instrument that delivers 50 microliters of luciferase enzyme reagent (Promega) to each well and then measures the resulting light omitted (integrating light emission from the wells for a 15 second period). The light emission is expressed as Relative Light Units (RLU) for each well. The measured RLU by the instrument is then exported to a Compaq computer and analyzed with software designed to provide analysis of the RLU of the beta-estradiol standard, subtraction of blank responses and interpolation of unknown responses to the standard curve.

A sample template for the 96 well plate analysis includes, the B-estradiol standard, test chemicals for analysis of potential estrogenic activity, and measurements of extracts of environmental chemicals for luciferase activity as well as control samples of background or solvent blank in the system. As described earlier, we have determined that the responsiveness of the BG1Luc4E2 cells is extremely sensitive to an edge effect in which determinations made in the outer wells of the plate are extremely variable and result in reduced confidence of analysis of luciferase activity in these wells. Therefore, on a 96 well plate 56 wells of the plate are useful for determination of estrogen dependent induction of luciferase activity. The ten standard dilutions of beta-estradiol are therefore applied to wells B2 through E3. This provides a standard curve of beta-estradiol in a two fold dilution series from 50 pg/ml down to 0.097 pg/ml. Solvent or blank controls are applied to a number of wells of the plate to provide replicate of estimates of the background response expected for luciferase expression by the BG1Luc4E2 cells (wells F3, G3, B4, F10, G10).

We have determined that the output of receptor mediated gene expression systems is best estimated by a 4 parameter Hill equation. Input of the RLU for samples is entered into this equation and the pg of estrogenic like activity for the sample is estimated from the model. The output is expressed as pg of estrogenic activity derived from the model.

The output of the analysis corrected for the amount of sample extracted for the determination. The estimated estrogenic activity of each sample is corrected for the dilution of sample extract that was used in the analysis. This analysis also displays a non-modeled graphic display of the data. The figure provides a graphic display of the output from the B-estradiol standard and a test chemical.

2.0 Dose-selection procedures, including the need for any dose range-finding studies or acute toxicity data prior to conducting the test:

The dose selection for Beta-estradiol standard is based upon the responsiveness of our genetically engineered BG1Luc4E2 cells to estrogen. The cells are extremely sensitive to estrogen and estrogen-like chemicals demonstrating a significant response to as little as 0.39 pg/ml solution of beta-estradiol. The BG1Luc4E2 cells respond with a dose dependent induction of luciferase activity up to a maximal concentration of 50 pg/ml.

A screening testing for estrogenic activity of a chemical is performed by initially performing a dose range finding experiment with the chemical. Ten milligrams of a pure chemical for testing of estrogenic activity is weighed out into glass vial and dissolved in one-milliliter of DMSO. A 10 fold dilution series is of the chemical is then produced by adding 10 microliters of the test compound to 90 microliters of DMSO in a 13 mm glass tube and repeating this procedure for six dilutions creating a dilution series of 1 mg/ml down to 1 ng/ ml. Four micoliters of these solutions is then added to 400 microliters of media (final concentrations of 10 micrograms/ml down to 10 picrograms/ml) and applied to the BG1Luc4E2 cells to evaluate induction of luciferase activity. Using this screening format 8 compounds can be evaluated per plate of BG1Luc4E2 cells. If a test chemical is positive for induction of luciferase activity a second experiment using a two fold dilution series at the concentrations that are active is performed. An example two-fold analysis of the activity of diethlstilbesterol was included in the example analysis provided from 50 pg/ml down to a concentration of 1.56 pg/ml.

3.0 Endpoint(s) measured:

The endpoint measured is the induction of luciferase activity in a human ovarian carcinoma, BG-1 that has been genetically engineered with a reporter gene construct that expresses the enzyme luciferase in response to exposure of the BG1Luc4E2 cell line to estrogen or estrogen-like chemicals.

4.0 Duration of exposure:

The duration of exposure to B-estradiol to induce maximal expression of the luciferase reporter gene in our BG1Luc4E2 bioassay is 24 hours. A significant induction of estrogen dependent expression of luciferase activity can be measured as early as two hours after exposure of the cells with half maximal induction occurring at eight hours following exposure of the BG1Luc4E2 cells [Rogers, 2000 #197].

5.0 Known limits of use:

The only known limits of use of the BG1Luc4E2 bioassay for measuring estrogen dependent induction of luciferase activity is if the chemical or environmental extract is toxic to the cellular

system. Toxicity could potentially inhibit induction of estrogen-dependent induction of luciferase activity. However, overt toxicity is assessed in the system by visual observation of the cells before measurement of luciferase induction. The sensitivity and large dynamic range of the BG1Luc4E2 bioassay system allows for dilution of the sample test compound to limit toxicity and yet estimate potential induction of estrogen-dependent luciferase expression.

6.0 Nature of the response assessed:

The response that is measured is the enzymatic activity of luciferase that is induced in our genetically engineered cells BG1Luc4E2 that express this enzyme in response to exposure to estrogen and estrogen-like chemicals. The enzyme activity is assessed by the production of light in a luminometer following addition of enzyme reagents.

7.0 Appropriate vehicle, positive, and negative controls and the basis for their selection:

The vehicle used for application of chemicals is DMSO. The response from the vehicle is the negative control for chemicals and solvent for extraction of environmental samples is the vehicle in testing environmental extracts. The positive control is B-estradiol which is the hormone ligand for the estrogen receptor.

8.0 Acceptable range of vehicle, positive and negative control responses:

Criteria have not been established for the range of vehicle, positive and negative control responses as yet since this system is in development. However, control charts are being established for responses. Generally, the vehicle response should be less than 20% of the maximal induction of the positive control at this time in development.

9.0 Nature of the data to be collected and the methods used for data collection:

The data collected are measurements of the light induction produced by the luciferase enzyme and are measured as relative light units detected by a luminometer. The data are stored as electronic files in a computer system that is backed up daily. They are secured in the laboratory and follow methods described in EPA method 2185: Good Automated Laboratory Practices.

10.0 Type of media in which data are stored:

The data are stored electronically in a Windows NT network. The network hard disk is backed up every 24 hours on a Compaq workstation.

11.0 Measures of variability:

In the screening mode of the assay replicate analysis are not performed, however the use of a varying doses of compound allows an estimate if the response demonstrates a trend. In confirmation assays, triplicate analysis is performed and statistical model testing can be performed on this data.

12.0 Statistical or non-statistical method(s) used to analyze the resulting data (including methods to analyze for a dose-response relationship). The method(s) employed should be justified and described:

The data that is generated from the B-estradiol standard is modeled using a four parameter Hill equation. The Hill equation is a mathematical model that generates the best fit for receptor mediated induction of gene expression (Kohn, Lucier et al. 1993; Kohn, Sewall et al. 1996; Kohn, Walker et al. 2001).

13.0 Decision criteria or the prediction model used to classify a test chemical (e.g., positive, negative, or equivocal), as appropriate:

There have been three initial criteria adopted for assigning a positive designation for a chemical in the BG1Luc4E2 estrogen screen. The first criteria is that the chemical induces luciferase activity that is greater than 3 times the standard deviation of the DMSO blank at an applied concentration of 10 micrograms/ml (designated +). The second more restrictive criteria are that the chemical induces BG1Luc4E2 bioassay system at both 10 and 1 microgram/ml (designated as ++). The third criteria is that the chemical induces luciferase activity at a number of concentrations in a two-fold dilution re-analysis demonstrating dose-dependent induction of luciferase and a relative response to B-estradiol can be assigned (designated +++). A negative designation for activity in the BG1Luc4E2 bioassay estrogen screen is assigned when no induction of luciferase activity is detected at any concentration over 3 times the standard deviation of the DMSO blank.

14.0 Information that is included in a test report.

Information in test reports include the standard curve generated by a two-fold dilution series of the positive control chemical B-estradiol, background determinations of solvent carrier (DMSO), modeling of the B-estradiol response using a four parameter Hill equation, and response of at six different 10 fold dilutions from 10 micrograms/ml down 10 picrograms/ml in our BG1Luc4E2 bioassay.

15.0 References

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Appendix B8

Protocol for the MVLN Assay

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Introduction

The purpose of this method is to characterize the estrogen activity of test chemicals. The assay utilizes an MCF-7[2] derivative that has been stably transfected with the Vit-Luc reporter gene[1]. Thus, the MVLN cell line expresses the endogenous estrogen receptors of MCF-7 and at the same time, contains an exogenous estrogen responsive reporter gene (luciferase). Therefore, the estrogen specific transcription activity of a test chemical is directly related to the luciferase measured in the lysate of treated MVLN cells. The MVLN assay procedure presented here is a modified version of published methods[1, 3]. While this protocol uses the MVLN subclone of MCF-7, all tissue culture materials, such as media and sera, are commercially available. In brief, MVLN cells are seeded into 96 well plates, fed media containing treatment compounds and then two days later, cell lysates are harvested and evaluated for luciferase activity. This general method should also work with other cell lines stably transfected with estrogen responsive reporter systems. When cell counting is called for in this procedure, consult the method "Monolayer Cell Counting with a Coulter Counter" from this laboratory.

Maintenance of Cell Stocks

The MVLN cell line must be obtained from their source[1]. The MVLN clone has been shown to maintain a stable, estrogen responsive phenotype in this laboratory over many passages (at least 50). Stock cultures should be maintained in 10% fetal bovine sera (FBS) media under 5% CO_2 in a 37° incubator. Such culture conditions will be "estrogen rich" and tend to favor cells that require estrogen for growth (MCF-7, MVLN). A regular schedule of passing stocks weekly is recommended. Monday pass into 6 T-25 flasks at a density of 1.5×10^6 for cells to be withdrawn and used for experiments. At the same time, seed 2 flasks at 8.0×10^5 for stock cells. This will be provide enough cells 7 days later to seed 2-3 96 well plates and another round of stocks. MVLN cells may grow slower than other MCF-7 derivatives. In addition, MVLN cells are very sensitive to seeding density. If seeded too light, MVLN cells will grow exceedingly slow and may not thrive. The common pH indicator phenol red has been shown to be estrogenic and therefore should not be used in MVLN cell cultures.

For routine passage, the MVLN cell monolayer is removed with trypsin/EDTA treatment. First, count one duplicate flask. Second, remove media from other flask(s). Then, wash each flask 3X with Ca^{++} free HBSS, remove and then add 2 ml trypsin for 5 minutes @ 37°. After incubation, dilute trypsin to 10 ml with whole media. To disperse the cells, use a sterile cannula-syringe (14 gauge, blunt tip, Luer lock needle with 1 cm at the tip bent 30 to 45°), draw the 10 ml of media up into the 10 ml syringe. Expel the media, with moderate force, through the bent cannula, towards the cell monolayer with a circular motion covering the cell growing surface of the flask. Repeat for a total of 3-5 cycles making sure that all the monolayer has been removed from the flask (keep air bubbles to a minimum). After last cycle, leave the cell suspension in the flask. Then, with a 10 ml pipette, rinse down the inside of the flask 5X with the cell solution.

An aliquot of this concentrated media-cell solution should then be diluted with media in a sterile vessel, mixed and the final volume used to seed flasks/plates. For precise seeding, it is recommended that the entire volume of cells and media to seed all the flasks/plates is mixed in a single vessel. For example, to seed 4 flasks with 1.5×10^6 cells each, make 25 ml of a seeding solution (5ml extra) that is 3.0×10^5 cells/ml, mix well and then add 5 ml to each T-25 flask. The goal of this method is to seed all flasks/plates identical.

Since all MVLN cells can express the reporter gene in response to estrogen, precision within MVLN assays is largely dependent on uniform seeding of plates/wells. It may be a good idea to practice seeding flasks and then count them the next day to check seeding performance.

MVLN Assay Setup and Time Sequence

With the following exceptions, passing MVLN cells for estrogen assays should be done as described above. It is essential that cells used to seed experimental plates have been withdrawn from estrogen 5 days prior to passage. In the example above, a stock flask of MVLN cells grown in FBS media are used to seed 6 T-25 flasks at a density of 1.5 x 10^6 cells/flask (to be withdrawn) and 2 flasks at 8.0 x 10^5 cells/flask (stocks) in 10% FBS media on Monday, one week prior to seeding experimental plates. When seeding MVLN stocks or experiments, one of the duplicate flasks is counted to determine the cells per flask count for that series (1 of 2 stocks is counted, 1 of 6 withdrawn flasks is counted). The day after seeding, "stock" flasks (8 x 10^5 cells/flask) are feed with the same 10% FBS media and fed 5 ml 10% FBS media every other day until used to seed more stock and withdrawn flasks the following week. The remaining 6 flasks are to be withdrawn from estrogens for one week and then passed into plates for experiments. The "withdrawn" flasks are fed the day after seeding with 5 ml 10% DCC FBS media which is almost devoid of estrogens. Two days after seeding, the 6 "withdrawn" flasks are withdrawn from estrogen by rinsing 3X with sterile PBS (all flask surfaces) and fed 5ml of 10% DCC FBS media. This PBS wash process is also repeated on days 3 to 4 after seeding and then these cells are kept on DCC media until used for seeding an MVLN experiment. When using the cannula to disperse cells after trypsin treatment, different cannulas and syringes must be used for withdrawn and stock cells or estrogens will contaminate the withdrawn cells. Keep in mind that it takes a week to get cells ready for a MVLN experiment.

Plates for experiments (96 well) are seeded on day 0 (Monday) with 8 x 10^4 cells/well in 100ul using 10% DCC FBS media. To ensure uniform seeding, mix the required cells and media in a sterile bottle. Seed the wells using the electronic pipetter set for dispensing 8, 100 µl aliquots. Dispense 100 µl in each well of one plate column with the pipet tip touching the side of the well. Repeat for all 12 columns in each 96 well plate. Mix the cell dilution bottle well before each fill of the pipet for seeding!

Day 1, (Tuesday), cells are fed treatment media. We recommend 4 wells per treatment dose. Treatment media is 10% DCC FBS into which treatments in ethanol carrier have been added. Treatment solutions (2 ml) may be made up in 5 ml polypropylene tubes (do not use polycarbonate or polystyrene tubes![4]) and should be no more than 0.1% v/v ethanol carrier solvent. Higher levels of ethanol may have confounding effects on MVLN studies. Treatment carrier solvents such as DMSO and methanol should be avoided since they may be toxic to cells and/or could have confounding effects on MVLN studies. If DMSO stock solutions must be used, be sure to have proper positive and negative controls in DMSO as well (see below). Media is removed from plate wells using a sterile 8 channel aspirator set with rubber bumpers set such that media only is removed from wells (no cells). Take care to remove media from only 1-3 columns at a time to prevent cells from drying out while adding treatments. Dose wells (100ul/well) using the electronic pipetter set at 4, 100 µl aliquots.

Experimental cells are dosed again the next day (day 2, Wednesday) using the same treatment solutions.

On day 3 (Thursday), treated cells are lysed for luciferase assay. First remove treatment media from each well using a nonsterile, 8 channel aspirator. Then, wash each well 2X with 50 ul PBS. It is essential that all PBS is removed from each well with the aspirator after this step. Thus, removal of the PBS by aspiration is followed by a 1 minute bench top incubation with the plate tipped 45°and a final aspiration of drained residue. To lyse cells, add 25 µl lysis buffer to

each well using the electronic multichannel pipetter and then incubate at room temperature for at least 30 minutes that includes at least 20 minutes on the rotating plate shaker set at 8.

MVLN Assay Design

Properly designed MVLN assays can be utilized to answer only the following 4 questions:

1. Does the test compound stimulate estrogen receptor mediated transcription (what is the shape of the corresponding dose response curve)?
2. If the test compound stimulates transcription, is this response through estrogen receptor mediated mechanisms (is the compound an estrogen receptor agonist)?
3. Can the test compound block the agonist effects of E_2 (is the compound an antiestrogen)?
4. Is the test compound toxic to MVLN cells?

Attempts to obtain additional information from the MVLN assay may be misleading. Keep in mind that this assay does not necessarily determine if the test compound binds to the ER.

Each data point of the MVLN assay should be run in quadruplicate during a trial (4 wells). Then, that same trial should be repeated at least two more times (total of 3 trials).

Example Experiment Setup

Blank (ethanol, same vol. as test cmpd.)) ····· 4 wells

E_2 (positive controls) 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7} M ····· 24 wells

ICI-182,780 10^{-7} M (check of estrogen free conditions) ····· 4 wells

E2 10^{-9} M + ICI-182,780 10^{-7} M (Check of ICI) ····· 4 wells

Test compound A: 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M ····· 16 wells

Test compound B: etc.

ICI 10^{-7} M + Test Comp. A 10^{-5} M (ER mechanism test) ····· 4 wells

ICI 10^{-7} M + Test Comp. B 10^{-5} M etc.

E2 10^{-9} M + Test Comp. A 10^{-5} M (antiestrogen test) ····· 4 wells

E2 10^{-9} M + Test Comp. B 10^{-5} M etc.

to a Total of 96 wells

Note1: Test compound toxicity is determined by comparing Luc activity of test compound treatments and/or test compound with ICI to blank and ICI alone.

Note2: When running more than one plate, the above controls should be used on at least one plate while other plates need only Blank, E_2 (positive controls) 10^{-10}, 10^{-9}M, ICI-182,780 10^{-7} M and E2 10^{-9} M + ICI-182,780 10^{-7} M.

Example Schedule

Day 0 (Monday)	Pass stocks, experimentals plated (from last weeks withdrawn cells)
Day 1 (Tuesday)	Feed all stock flasks (stock and withdrawn for next week) Dose experimental plates
Day 2 (Wednesday)	Withdraw stocks (for next week) Dose experimental plates

Day 3 (Thursday)	Harvest plates and Run Luc Assay
	Feed/withdraw stocks (for next week)
Day 4 (Friday)	Feed/withdraw stocks (for next week)

Regarding an Estrogen Free Laboratory Environment

All glassware, caps, hoses, etc. that may contact media must be free of estrogenic compounds. Soap wash (1% Liqui-Nox), 3X hot water rinse, 3X rinse with ddH_2O, air dry, rinse with 95% ethanol, air dry and then autoclave bottles with caps loosened. Glassware may also be baked at 250° C for 12-24 hrs after ethanol wash. Your cell culture environment should be characterized for estrogen contamination with the MVLN assay treated with and without added ICI-182,780[5]. If the "estrogen free" cells treated with only media have more Luc activity (> 10%) than the ICI treated cells, you have estrogen contamination. All experiments conducted in the presence of such contamination are suspect since regardless of how they are set up, you are testing combinations of estrogenic chemicals. We have found plastic vessels and implements to be the major source of estrogen contamination. Polystyrene and polycarbonate seem to be the big problems[4]. Do not use culture flasks with "phenolic" caps. Filter units may also add estrogenic substances to media. The Corning bottle top units (orange) are suspect. Zap Caps seem to add some kind of nonestrogenic mitogen which induces MCF-7 cells to grow at maximum rate, even in the presence of ICI. It is unclear what effect Zap Cap contamination has on MVLN assays. Also, it appears to be relatively easy to extract estrogens from gloves and/or the hands of females when rinsing items with ethanol. Lastly, ethanol rinsed vessels and implements must be thoroughly dry before use in making media or other procedures.

Media

1. DMEM powder for 10L, (phenol red free, Mediatech 90-013-PB).

k. 59.58 gm HEPES (Gibco 11344-033), media will be 20 mM.

l. 37 gm $NaHCO_3$

3. 100 ml non-essential amino acids (Gibco 11140-019), media will be 0.1 mM.

m. 100 ml sodium pyruvate (Gibco 11360-070), media will be 1 mM.

n. 200 ml L-Glutamine (Gibco 25030-081)

6. 1.0 ml/L media Gentamicin (Gibco 15750-011).

In 2 L tissue culture grade water, add 1 & 2 above. Mix 30 minutes in 3 L beaker. Add 3 & mix 10 minutes, pH to 7.2. Transfer media with 2 L graduate to large mixing bottle and dilute media to total volume of 10 L by quantitatively transferring and washing residue from beaker. Mix 15 min. Check pH and adjust as required. Filter 450 ml into each 500 ml sterile bottle (Gelman VacuCap 4622 or Gelman Micro Culture Capsule 12158). Store media at 4° C.

One 500 ml bottle of media ready to be use on MVLN cells contains: 450 ml DMEM (from above), 5 ml each of non-essential amino acids and sodium pyruvate solutions, 10 ml L-Glutamine solution, 0.5 ml Gentamicin and 50 ml FBS or DCC FBS.

Sera

FBS	Hyclone Characterized Fetal Bovine Sera (A-1115-L)
DCC FBS	Hyclone Charcoal/Dextran Fetal Bovine Sera (A-1120-L)

Buffers

Ca^{++} Free HBSS	Gibco 14185-052
PBS	Gibco 14080-055
Lysis Buffer	Promega E153A

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Appendix C

Chemical and Product Class Information for the Substances Tested in the *In Vitro* ER TA Assays

Information Sorted by Chemical Name

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Information Sorted by Substance Name

Substance Name	Synonyms	CASRN	Chemical Class	Product Class
Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
Acenaphthene		83-32-9	Polycyclic aromatic hydrocarbon	Chemical intermediate
Acenaphthylene		208-96-8	Polycyclic aromatic hydrocarbon	Chemical intermediate
6-Acetyl-1,1,2,4,4,7-hexamethyltetraline	AHTN; Tonalid	21145-77-7	Polycyclic aromatic hydrocarbon	Fragrance ingredient
Acrinathrin		101007-06-1	Pyrethroid ester	Pesticide
Alachlor		15972-60-8	Anilide	Pesticide
Aldicarb		116-06-3	Carbamate	Pesticide
Aldosterone		52-39-1	Steroid, nonphenolic	Pharmaceutical
Allenolic Acid	R26008	553-39-9	Polycyclic aromatic hydrocarbon	
δ-*trans* -Allethrin	Allethrin; 2-Cyclopenten-1-one, 2-allyl-4-hydroxy-3-methyl-, 2,2-dimethyl-3-(2-methylpropenyl) cyclopropanecarboxylate	584-79-2	Pyrethrin	Pesticide
2-Aminoestratriene-3,17β-diol		107900-30-1	Steroid, phenolic	
4-Aminoestratriene-3,17β-diol		107900-31-2	Steroid, phenolic	
1-Aminoestratrien-17β-ol			Steroid, nonphenolic	
2-Aminoestratrien-17β-ol		17522-06-4	Steroid, nonphenolic	
3-Aminoestratrien-17β-ol			Steroid, nonphenolic	
4-Aminoestratrien-17β-ol		17522-04-2	Steroid, nonphenolic	
Aminotriazole	Amitrole	61-82-5	Azole	Pesticide
4-*tert* -Amylphenol	4-*tert* -Pentylphenol	80-46-6	Alkylphenol	Chemical intermediate
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
4-Androstenediol	3β,17β-Dihydroxy-4-androstene; Androst-4-ene-3β,17β-diol	1156-92-9	Steroid, nonphenolic	Pharmaceutical
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-Androstenedione	63-05-8	Steroid, nonphenolic	
Anthanthrene		191-26-4	Polycyclic aromatic hydrocarbon	
Anthracene		120-12-7	Polycyclic aromatic hydrocarbon	
Apigenin	4',5,7-Trihydroxyflavone	520-36-5	Flavone	Natural product
Aroclor 1016		12674-11-2	Polychlorinated biphenyl	Dielectric fluid
Aroclor 1221		11104-28-2	Polychlorinated biphenyl	Dielectric fluid
Aroclor 1232		11141-16-5	Polychlorinated biphenyl	Dielectric fluid
Aroclor 1242		53469-21-9	Polychlorinated biphenyl	Dielectric fluid
Aroclor 1248		12672-29-6	Polychlorinated biphenyl	Dielectric fluid

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
Aroclor 1254		11097-69-1	Polychlorinated biphenyl	Dielectric fluid
Aroclor 1260		11096-82-5	Polychlorinated biphenyl	Dielectric fluid
Aroclor 1268		11100-14-4	Polychlorinated biphenyl	Dielectric fluid
Atrazine		1912-24-9	Triazine; Aromatic amine	Pesticide
Baygon	Propoxur	114-26-1	Carbamate	Pesticide; Pharmaceutical (veterinary)
Bendiocarb		22781-23-3	Carbamate	Pesticide
Benomyl		17804-35-2	Carbamate; Imidazole	Pesticide
2,3-Benzanthracene	Naphthacene; Tetracene	92-24-0	Polycyclic aromatic hydrocarbon	
Benz[*a*]anthracene	1,2-Benzanthracene	56-55-3	Polycyclic aromatic hydrocarbon	
Benzo[*a*]carbazole	1,2-Benzcarbazole	239-01-0	Carbazole; Aromatic amine	Component of crude oils
Benzo[*c*]carbazole			Carbazole; Aromatic amine	Component of crude oils
Benzo[*b*]fluoranthene	Benz[*e*]acephenanthrylene	205-99-2	Polycyclic aromatic hydrocarbon	
Benzo[*j*]fluoranthene		205-82-3	Polycyclic aromatic hydrocarbon	
Benzo[*k*]fluoranthene		207-08-9	Polycyclic aromatic hydrocarbon	
Benzo[*b*]fluorene	2,3-Benzofluorene	243-17-4	Polycyclic aromatic hydrocarbon	
Benzo[*b*]naphtho[*2,1-d*]thiophene	1,2-Dibenzodiphenylene sulfide	239-35-0	Polycyclic aromatic hydrocarbon; Thiophene	
Benzo[*b*]naphtho[*2,3-d*]thiophene		243-46-9	Polycyclic aromatic hydrocarbon; Thiophene	
Benzo[*ghi*]perylene	1,12-Benzperylene	191-24-2	Polycyclic aromatic hydrocarbon	
Benzo[*c*]phenanthrene	3,4-Benzophenanthrene	195-19-7	Polycyclic aromatic hydrocarbon	
Benzophenone		119-61-9	Benzophenone	Pharmaceutical
Benzophenone-1		131-56-6	Benzophenone	UV light absorber (sunscreens, polymers)
Benzophenone-12	(2-hydroxy-4-(octyloxy)phenyl)phenylmethanone; Octabenzone	1843-05-6	Benzophenone	Pharmaceutical (excipient)
Benzophenone-2	2,2',4,4'-Tetrahydroxybenzophenone	131-55-5	Benzophenone	Enzyme inhibitor
Benzophenone-3	2-Hydroxy-4-methoxybenzophenone; Oxybenzone; Eusolex 4360	131-57-7	Benzophenone	UV light absorber (sunscreens and resins)
Benzophenone-4	2-Hydroxy-4-methoxybenzophenone-5-sulfonic acid; Sulisobenzone	4065-45-6	Benzophenone	UV light absorber (sunscreens)
Benzophenone-6	Bis(2-hydroxy-4-methoxyphenyl)methanone	131-54-4	Benzophenone	UV light absorber (paints and plastics)
Benzophenone-7	Chlorohydroxy benzophenone; 5-Chloro-2-hydroxybenzophenone	85-19-8	Benzophenone	UV light absorber
Benzophenone-8	Dioxybenzone; 2,2'-Dihydroxy-4-methoxybenzophenone	131-53-3	Benzophenone	
Benzo[*a*]pyrene	3,4-Benzopyrene	50-32-8	Polycyclic aromatic hydrocarbon	

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
Benzo[*e*]pyrene	1,2-Benzopyrene; 4,5-Benzopyrene	192-97-2	Polycyclic aromatic hydrocarbon	
Benzo[*a*]pyrene-1,6-dione		3067-13-8	Polycyclic aromatic hydrocarbon	
Benzo[*a*]pyrene-3,6-dione		3067-14-9	Polycyclic aromatic hydrocarbon	
Benzo[*a*]pyrene-6,12-dione		3067-12-7	Polycyclic aromatic hydrocarbon	
Benzylparaben	Benzyl 4-hydroxybenzoate; Benzyl *p* -hydroxybenzoate	94-18-8	Paraben	
Benzyl salicylate	Salicyclic acid benzyl ester	118-58-1	Salicylic acid	Fixative (perfumes and sunscreens)
Betulin	Trochol	473-98-3	Polycyclic hydrocarbon	Pharmaceutical
Bifenix	5-(2,4-Dichlorophenoxy)-2-nitrobenzoic acid methyl ester; Bifenox	42576-02-3	Organochlorine	Pesticide
Biochanin A	4'-Methylgenistein	491-80-5	Isoflavone	
2,2'-Biphenol	2,2'-Dihydroxybiphenyl	1806-29-7	Biphenyl; Phenol	
Biphenyl		92-52-4	Biphenyl	Pesticide
3,4-Biphenyldiol			Biphenyl	
4,4'-Biphenyldiol	4,4'-Dihydroxybiphenyl	92-88-6	Biphenyl; Phenol	Antioxidant; Chemical intermediate (coatings and adhesives)
2-Biphenylol	2-Hydroxybiphenyl; 2-Phenylphenol	90-43-7	Biphenyl; Phenol	Pesticide; Disinfectant; Preservative; Plasticizer; Chemical intermediate
3-Biphenylol	3-Hydroxybiphenyl; 3-Phenylphenol	580-51-8	Biphenyl; Phenol	Chemical intermediate
4-Biphenylol	4-Hydroxybiphenyl; 4-Phenylphenol	92-69-3	Biphenyl; Phenol	Chemical intermediate
Bis(2-*n*- butoxyethyl) phthalate	DBoEP	117-83-9	Phthalate	Plasticizer
Bisdesoxyestradiol	1,3,5(10)-Estratriene; Estratriene; Bisdesoxy E2	1217-09-0	Steroid, nonphenolic	
Bis(ethoxyethyl) phthalate	DEoEP	605-54-9	Phthalate	
Bis[2-(2-ethoxyethyoxy)ethyl] phthalate	DEoEoEP		Phthalate	
Bis(2-ethylhexyl)adipate	DEHA; Dioctyl adipate	103-23-1	Dicarboxylic acid	Plasticizer
Bis(2-ethylhexyl) hexahydrophthalate	DEHhP		Phthalate	
Bis(2-ethylhexyl)isophthalate	Dioctyl isophthalate	137-89-3	Phthalate	
Bis(2-ethylhexyl) phthalate	Diethylhexylphthalate; DEHP	117-81-7	Phthalate	Plasticizer
Bis(4-hydroxyphenyl)ethane		6052-84-2	Bisphenol	Chemical intermediate
Bis(4-hydroxyphenyl)methane	4,4'-methylenediphenol	620-92-8	Bisphenol	Pharmaceutical
2,2-Bis(*p* -hydroxyphenyl)-1,1,1-trichloroethane	HPTE; Dihydroxymethoxychlor; 2,2-bis(4-hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	Organochlorine; Bisphenol	Pesticide metabolite
Bis(methoxyethyl) phthalate		117-82-8	Phthalate	
Bisphenol A	4,4'-Isopropylidinediphenol; 4,4'-(1-Methylethylidene)bisphenol	80-05-7	Bisphenol	Chemical intermediate
Bisphenol A dimethacrylate	2,2-Di(4-methacryloxyphenyl)propane	3253-39-2	Acrylate; Bisphenol	Chemical intermediate

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
Bisphenol A glucuronide			Bisphenol; Glucoronide conjugate	
Bisphenol B	2,2-Bis(4-hydroxyphenyl)butane	77-40-7	Bisphenol	Chemical intermediate
6-Bromo-2-naphthol		15231-91-1	Polycyclic aromatic hydrocarbon; Phenol	Pharmaceutical
Bromopropylate	Isopropyl 4,4'-dibromobenzilate	18181-80-1	Diphenylacetic acid	Pesticide
2-Bromo-4-(2,4,6-tribromophenoxy)phenol			Brominated aromatic hydrocarbon	
4-Butoxyphenol	4-*n* -Butoxyphenol	122-94-1	Alkylphenol	
Butylate	S-Ethyldiisobutyl thiocarbamate	2008-41-5	Carbamate	Pesticide
Butylated hydroxyanisole	BHA; (1,1-Dimethylethyl-4-methoxyphenol	25013-16-5	Phenol; Ether	Preservative (foods, cosmetics, pharmaceuticals)
Butylated hydroxytoluene	BHT	128-37-0	Phenol	Preservative (foods and cosmetics)
***n* -Butylbenzene**	1-Phenylbutane	104-51-8	Aromatic hydrocarbon	
Butyl benzyl phthalate	Benzyl butyl phthalate; n-Butyl benzyl phthalate; Butylbenzyl phthalate ester	85-68-7	Phthalate	Plasticizer
4-*tert* -Butylcatechol		98-29-3	Catechol	Antioxidant (foods and cosmetics); Chemical intermediate; Antimicrobial agent
Butyl cyclohexyl phthalate	BCHP	84-64-0	Phthalate	Plasticizer
Butyl decyl phthalate	BDcP; 1,2-Benzenedicarboxylic acid, butyl decyl ester	89-19-0	Phthalate	
Butyl 2-ethylhexyl phthalate	BEHP; 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	85-69-8	Phthalate	Plasticizer
5-*tert* -Butyl-4-hydroxy-2-methyl-phenyl sulfide	4,4'-Thiobis(6-*tert* -butyl-3-cresol); Santonox	96-69-5	Cresol	Antioxidant (rubber, latex, food packaging, vinyl polymers)
Butyl isodecyl phthalate	BIDP	42343-36-2	Phthalate	
Butyl methoxydibenzoylmethane	Eusolex 9020; Parsol 1789; Avobenzone	70356-09-1	Benzoate	Sunscreen
Butyl octyl phthalate	BOP	84-78-6	Phthalate	
Butylparaben	Butyl 4-hydroxybenzoate; Butyl p-hydroxybenzoate	94-26-8	Paraben	Food additive; Pharmaceutical additive
2-*tert* -Butylphenol		88-18-6	Phenol	Chemical intermediate (resins, plasticizers, perfumes and other products)
3-*tert* -Butylphenol	3-(1,1-Dimethylethyl)phenol	585-34-2	Phenol	
4-*sec* -Butylphenol	4-(1-Methylpropyl)phenol	99-71-8	Phenol	Pharmaceutical
4-*tert* -Butylphenol	4-(1,1-Dimethylethyl)phenol; Butylphen	98-54-4	Phenol	Chemical intermediate (coatings); Lubricant additive; Antioxidant (soap)
Caffeine	1,3,7-Trimethylxanthine	58-08-2	Purine	Pharmaceutical; Food additive
Captan	N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide	133-06-2	Indole	Pesticide; Preservative (cosmetics)
Carbaryl	1-Naphthyl methylcarbamate	63-25-2	Carbamate; Polycyclic aromatic hydrocarbon	Pesticide
Carbazole		86-74-8	Pyrrole	Chemical intermediate

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
Carbofuran	Furadan	1563-66-2	Carbamate	Pesticide
Carbosulfan	Dibutylaminosulfenylcarbofuran	55285-14-8	Carbamate	Pesticide
Carvacrol	1-Hydroxy-2-methyl-5-isopropylbenzene	499-75-2	Phenol	Pharmaceutical; Flavoring; Perfumes; Disinfectant
Catechol		120-80-9	Phenol	Antioxidant
Chlordane		57-74-9	Organochlorine	Pesticide
α-Chlordane	*cis* -Chlordane	5103-71-9	Organochlorine	Pesticide
γ-Chlordane	*trans* -Chlordane	5103-74-2	Organochlorine	Pesticide
Chlordimeform	Chlorphenamidine	6164-98-3	Amidines; Organochlorine	Pesticide
Chlorfenvinfos	Birlane	470-90-6	Organophosphate; Organochlorine	Pesticide
Chlorobenzilate	Ethyl 4,4'-dichlorobenzilate	510-15-6	Organochlorine	Pesticide
2-Chlorobiphenyl	*o* -Chlorobiphenyl	2051-60-7	Organochlorine; Biphenyl	
4-Chlorobiphenyl	4-Chloro-1,1'-biphenyl	2051-62-9	Organochlorine; Biphenyl	
2'-Chloro-4-biphenylol			Biphenyl; Organochlorine	
2-Chloro-4-biphenylol		23719-22-4	Polychlorinated biphenyl	
4-Chloro-4'-biphenylol		28034-99-3	Biphenyl; Organochlorine	
4-Chloro-3,5-dimethylphenol	Chloroxylenol; 2-Chloro-5-hydroxy-1,3-dimethylbenzene	88-04-0	Phenol; Organochlorine	Antimicrobial agent
11β-Chloromethylestradiol		71794-60-0	Steroid, phenolic	
4-Chloro-2-methylphenol	4-Chloro-*o* -cresol	1570-64-5	Organochlorine	Chemical intermediate
4-Chloro-3-methylphenol	Chlorocresol; 4-Chloro-*m* cresol	59-50-7	Phenol; Organochlorine	Preservative (Glue, gum, paint, ink, leather); Pesticide
Chlorosulfuron		64902-72-3	Triazine; Benzenesulfonamide	Pesticide
Chlorothalonil	2,4,5,6-Tetrachloroisophthalonitrile; 1,3, Dicyanotetrachlorobenzane	1897-45-6	Nitrile; Organochlorine	Pesticide
Chlorothymol	4-Chloro-5-methyl-2-(1-methylethyl)phenol	89-68-9	Phenol; Organochlorine	Antimicrobial agent
Chlorpyrifos	Dursban	2921-88-2	Organothiophosphate	Pesticide
Cholesterol		57-88-5	Steroid, nonphenolic	Natural product (animal); Pharmaceutical
Chrysene	Benzo[*a*]phenanthrene; 1,2-Benzphenanthrene	218-01-9	Polycyclic aromatic hydrocarbon	
Chrysin	5,7-Dihydroxyflavone	480-40-0	Flavone	Natural product
Citrostadienol			Steroid, nonphenolic	Natural product
***trans* -Clomiphene**	*trans* -Clomiphene	911-45-5	Stilbene	Pharmaceutical
Cocobetaine		68411-97-2	Betaine	Surfactant (shampoo)
Coconut amido betaine			Betaine	
Corn oil		8001-30-7	Lipid	Food; Pharmaceutical (solvent)
Corticosterone	17-Deoxycortisol; 11,12-Dihydroxyprogesterone; 11β,21-Dihydroxyprogesterone; 11-Hydroxycorticoaldosterone	50-22-6	Steroid, nonphenolic	Pharmaceutical

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
Cortisol	Hydroxyprogesterone	50-23-7	Steroid, phenolic	Pharmaceutical
Coumestrol	2-(2,4-Dihydroxyphenyl)-6-hydroxy-3-benzofurancarboxylic acid delta lactone	479-13-0	Coumarin; Phenol	Natural product (phytoestrogen)
Creosote	Coal tar creosote	8001-58-9	Mixture	Preservative (wood)
***o* -Cresol**	2-Methylphenol	95-48-7	Phenol	Chemical intermediate; Solvent; Disinfectant
***p* -Cresol**	4-Methylphenol	106-44-5	Phenol	Chemical intermediate; Flavor; Solvent; Monomer
Cumene	Isopropylbenzene	98-82-8	Aromatic hydrocarbon	Solvent; Chemical intermediate
***p* -Cumylphenol**	4-(1-Methyl-1-phenylethyl)phenol	599-64-4	Alkylphenol	
Curcumin	Natural yellow	458-37-7	Catechol	Dye (food, cotton, wool, silk); Reagent (analytical); Stain (biological); Pharmaceutical
Cyanazine		21725-46-2	Triazine	Pesticide
Cycloprothrin		63935-38-6	Pyrethroid ester	Pesticide
Cyfluthrin		68359-37-5	Pyrethroid ester	Pesticide
Cyhalothrin		68085-85-8	Pyrethroid ester	Pesticide
***p* -Cymene**	*p* -Isopropyltoluene	99-87-6	Terpene	Chemical intermediate; Solvent
Cypermethrin	α-Cyano(3-phenoxyphenyl) methyl (+-)*cis,trans* -3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylate	52315-07-8	Nitrile; Organochlorine; Diphenyl ether	Pesticide
Cyproterone acetate	CPA; 1,2-α-Methylene-6-chloro-(sup 4,6)-pregnadiene-17α-ol-3,20-dione 17α-acetate	427-51-0	Steroid, nonphenolic	Pesticide
Dacthal	DCPA; Dimethyl 2,3,5,6-tetrachloroterephthalate	1861-32-1	Phthalate	Pesticide
Daidzein	4',7-Dihydroxyisoflavone	486-66-8	Isoflavone	Natural product (phytoestrogen)
Daidzin		552-66-9	Isoflavone	Pharmaceutical
***p,p′* -DDA**	2,2-Bis(*p* -chlorophenyl)acetic acid	83-05-6	Organochlorine	Pharmaceutical
***o,p′* -DDD**	Mitotane; 1,1-Dichloro-2-(o -chlorophenyl)ethane; *o,p′* -TDE; 2,4'-Dichlorodiphenyldichloroethane	53-19-0	Organochlorine; Diphenylalkane	Pesticide; Pharmaceutical
***p,p′* -DDD**	2,2-Bis(4-chlorophenyl)-1,1-dichloroethane; *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-(*o* -chlorophenyl)ethylene; 1,1-Dichloro-2-[2-chlorophenyl]	3424-82-6	Organochlorine; Diphenylalkane	Pesticide metabolite
***p,p′* -DDE**	1,1-Dichloro-2,2-bis(*p* -chlorophenyl)ethylene; 4,4-DDE	72-55-9	Organochlorine; Diphenylalkane	Pesticide metabolite
***o,p′* -DDT**	2-(*o* -Chlorophenyl)-2-(*p* -chlorophenyl)-1,1,1-trichloroethane	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; DDT	50-29-3	Organochlorine; Diphenylalkane	Pesticide
DDT (technical)		8017-34-3	Organochlorine	Pesticide

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Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
Decachlorobiphenyl	PCB 209	2051-24-3	Polychlorinated biphenyl	Dielectric fluid
2-*sec* -Decylphenol			Alkylphenol	
4-*sec* -Decylphenol			Alkylphenol	
Dehydroepiandosterone	Dehydroisandrosterone; Androstenolone	53-43-0	Steroid, nonphenolic	Pharmaceutical
Deltamethrin		52918-63-5	Pyrethroid ester	Pesticide
Desethylatrazine			Triazine	Pesticide degradation product
Desisopropylatrazine			Triazine	Pesticide degradation product
Dexamethasone		50-02-2	Steroid, nonphenolic	Pharmaceutical
Diamyl phthalate	DPeP	131-18-0	Phthalate	
Diazinon		333-41-5	Organothiophosphate	Pesticide
Dibenz[*a,c*]anthracene	1,2,3,4-Dibenzanthracene	215-58-7	Polycyclic aromatic hydrocarbon	
Dibenz[*a,h*]anthracene	1,2:5,6-Dibenzanthracene	53-70-3	Polycyclic aromatic hydrocarbon	
3,3'-Dibromobisphenol A	diBBPA		Bisphenol	Flame retardant (degradation product)
2,6-Dibromo-4-(2,4,6-tribromophenoxy)phenol			Undetermined	
2,4-Di-*tert* -butyl-6(5-chloro-2H-benzotriazol-2-yl)phenol		3864-99-1	Undetermined	
2,6-Di-*tert* -butyl-4-(dimethylamino-methyl)phenol		88-27-7	Alkylphenol	
4,6,-Di-*tert* -butyl-2,2'-ethylidene		35958-30-6	Phenol, Stilbene	
2,4-Dibutylphenol			Alkylphenol	
2,6-Dibutylphenol			Alkylphenol	
2,6-Di-*tert* -butylphenol		128-39-2	Alkylphenol	Chemical intermediate; Antioxidant (fuel)
Dibutyl phthalate	Di-*n* -butyl phthalate; DBP; Di-*n* -butyl phthalate ester; Dibutyl 1,2-benzenedicarboxylate	84-74-2	Phthalate	Plasticizer
Dicamba	3,6-Dichloro-2-methoxybenzoic acid	1918-00-9	Organochlorine	Pesticide
Dichlobenil	2,6-Dichlorobenzonitrile	1194-65-6	Organochlorine; Nitrile	Pesticide
2,5-Dichlorobiphenyl	PCB 9	34883-39-1	Polychlorinated biphenyl	Dielectric fluid
2,6-Dichlorobiphenyl	PCB 10	33146-45-1	Polychlorinated biphenyl	Dielectric fluid
3,5-Dichlorobiphenyl	PCB 14	34883-41-5	Polychlorinated biphenyl	Dielectric fluid
2,5-Dichloro-2',3'-biphenyldiol			Polychlorinated biphenyl	
2,5-Dichloro-3',4'-biphenyldiol			Polychlorinated biphenyl	
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	
3,5-Dichloro-2'-biphenylol			Polychlorinated biphenyl	
3,5-Dichloro-4'-biphenylol			Polychlorinated biphenyl	

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
Dichlorophen	2,2'-Dihydroxy-5,5'-dichlorodiphenylmethane	97-23-4	Organochlorine; Phenol	Pesticide; Antimicrobial agent; Pharmaceutical
2,4-Dichlorophenol		120-83-2	Organochlorine	Pesticide
2,4-Dichlorophenoxyacetic acid	2,4-D	94-75-7	Phenoxyacetic acid; Organochlorine	Pesticide; Plant growth regulator
4-(2,4-Dichlorophenoxy) butanoic acid	2,4-DB	94-82-6	Organochlorine	Pesticide
1,2-Dichloropropane		78-87-5	Chlorinated hydrocarbon	Pesticide
Diclofop-methyl	2-(4-(2,4-Dichlorophenoxy)phenoxy)propanoic acid methyl ester	51338-27-3	Organochlorine; Phenyl ether	Pesticide
Dicofol	1,1-Bis(chlorophenyl)-2,2,2-trichloroethanol	115-32-2	Organochlorine	Pesticide
Dicyclohexyl phthalate	DCHP	84-61-7	Phthalate	Plasticizer
Dieldrin		60-57-1	Organochlorine; Chlorinated cyclodiene	Pesticide
Dienestrol	*trans,trans* -Dienestrol	84-17-3	Diphenolalkene	Pharmaceutical
***cis,cis* -Diethyldihydroxytetrahydrochrysene**			Polycyclic aromatic hydrocarbon	
***R,R-cis,cis* -Diethyldihydroxytetrahydrochrysene**			Polycyclic aromatic hydrocarbon	
***S,S -cis ,cis* -Diethyldihydroxytetrahydrochrysene**			Polycyclic aromatic hydrocarbon	
Diethyl phthalate	DEP	84-66-2	Phthalate	Solvent; Plasticizer; Pesticide
Diethylstilbestrol	DES	56-53-1	Stilbene	Pharmaceutical
5,11-*trans* -Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon	
(5*R* ,11*R*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon	
(5*S* ,11*S*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol			Polycyclic aromatic hydrocarbon	
Dihexyl phthalate	DHP	84-75-3	Phthalate	Plasticizer
Dihydrogenistein	4',5,7-Trihydroxyisoflavan-4-one	21554-71-2	Isoflavone	Natural product
Dihydroglycitein	4*H* -1-Benzopyran-4-one, 2,3-dihydro-7-hydroxy-3-(4-hydroxyphenyl)-6-methoxy- (9CI)	94105-88-1	Isoflavone	Natural product
5α-Dihydrotestosterone	Androstanolone; Stanolone; 4-Dihydrotestosterone; 4,5α-Dihydrotestosterone	521-18-6	Steroid, nonphenolic	Pharmaceutical
4,4'-Dihydroxybenzophenone	Bis(4-hydroxyphenyl)ketone; *p,p* 'dihydroxybenzophenone	611-99-4	Benzophenone; Phenol	Pharmaceutical; Chemical intermediate (monomer)
4,5-Dihydroxybenzo[*a*]pyrene		37571-88-3	Polycyclic aromatic hydrocarbon	
7,8-Dihydroxybenzo[*a*]pyrene		60657-25-2	Polycyclic aromatic hydrocarbon	
9,10-Dihydroxybenzo[*a*]pyrene		58886-98-9	Polycyclic aromatic hydrocarbon	
4,4'-Dihydroxychalcone		3600-61-1	Chalcone	
Dihydroxy-DDE			Organochlorine	Pesticide metabolite
4',2-Dihydroxy-4,6-dimethoxydihydrochalcone			Chalcone	

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
4,4'-Dihydroxy-2,6-dimethoxydihydrochalcone			Chalcone	
4',7-Dihydroxyflavone		2196-14-7	Flavone	
Diisobutyl phthalate		84-69-5	Phthalate	Plasticizer
Diisodecyl phthalate		26761-40-0	Phthalate	Plasticizer
Diisoheptyl phthalate		41451-28-9	Phthalate	Plasticizer
Diisohexyl phthalate		71850-09-4	Phthalate	Plasticizer
Diisononyl phthalate		28553-12-0	Phthalate	Plasticizer
2,6-Diisopropylnaphthalene	DIPN	24157-81-1	Polycyclic aromatic hydrocarbon	
4,4'-Dimethoxybenzhydrol	*p,p'* -Dimethoxybenzhydryl alcohol	728-87-0	Alcohol; Aromatic hydrocarbon	
Dimethoxy-DDE			Organochlorine	Pesticide metabolite
7,12-Dimethylbenz[*a*]anthracene		57-97-6	Polycyclic aromatic hydrocarbon	
Dimethyl isophthalate	DMIP	1459-93-4	Phthalate	Plasticizer; Chemical intermediate
Dimethyl phthalate		131-11-3	Phthalate	Plasticizer; Solvent
Dimethyl sulfoxide		67-68-5	Sulfoxide	Pharmaceutical
Dimethylterephthalate		120-61-6	Pesticide	Insect repellent
(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	
2,4-Dinonylphenol		137-99-5	Phenol	Chemical intermediate; Solvent
Dinonyl phthalate	1,2-Benzenedicarboxylic acid dinonyl ester	84-76-4	Phthalate	Solvent
Dinoseb	4,6-Dinitro-2-*sec* -butylphenol	88-85-7	Nitrophenols	Pesticide
Di-*n* -octyl phthalate	DnOP	117-84-0	Phthalate	Plasticizer
Diosgenin		512-04-9	Steroid, nonphenolic	Natural product (phytosterol); Chemical intermediate; Pharmaceutical
1,4-Dioxane	1,4-Diethylenedioxide; 1,4-Dioxacyclohexane	123-91-1	Dioxane	Solvent; Adhesives; Cosmetics; Coatings
Diphenyl phthalate		84-62-8	Phthalate	
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	
Disulfoton		298-04-4	Organothiophosphate	Pesticide
Ditridecyl phthalate		119-06-2	Phthalate	Plasticizer
Diundecyl phthalate		3648-20-2	Phthalate	Plasticizer; Cosmetics
Diuron		330-54-1	Urea	Pesticide
Dodecyl gallate	Lauryl gallate	1166-52-5	Hydroxybenzoic acid	Preservative (foods); Antioxidant

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
Dodecylparaben	Dodecyl 4-hydroxybenzoate	2664-60-0	Paraben; Hydroxybenzoic acid	Pharmaceutical
4-*sec* -Dodecylphenol			Alkylphenol	
4-*sec* -Dodecylphenol dipropoxylate			Alkylphenol	
Dodemorph	4-Cyclododecyl-2,6-dimethylmorpholine	1593-77-7	Undetermined	Pesticide
Droloxifene	3-Hydroxytamoxifen	82413-20-5	Stilbene; Triphenylethylene	Pharmaceutical
EM-652	Ritetronium	37607-02-6	Piperidine	Pharmaceutical
α-Endosulfan		959-98-8	Organochlorine; Chlorinated cyclodiene	Pesticide
α,β-Endosulfan		115-29-7	Organochlorine; Chlorinated cyclodiene	Pesticide
β-Endosulfan		33213-65-9	Organochlorine; Chlorinated cyclodiene	Pesticide
Endrin		72-20-8	Organochlorine; Chlorinated cyclodiene	Pesticide
Endrin aldehyde		7421-93-4	Organochlorine; Chlorinated cyclodiene	
Epichlorohydrin	1,2-Epoxy-3-chloropropane	106-89-8	Cyclic ether	Chemical intermediate; Solvent; Monomer
Epidermal growth factor	Urogastrone	62229-50-9	Polypeptide	Pharmaceutical
17α-Epitestosterone	Androst-4-en-3-one, 17α-hydroxy-; Epitestosterone	481-30-1	Steroid, nonphenolic	
d-Equilenin	3-Hydroxyestra-1,3,5(10),6,8-entaen-17-one; d-Equilinen	517-09-9	Steroid, phenolic	Pharmaceutical
Equilin		474-86-2	Steroid, phenolic	Pharmaceutical
Equol	4',7-Dihydroxyisoflavan	531-95-3	Isoflavone	Pharmaceutical
17α-Estradiol	Estra-1,3,5(10)-triene-3,17α-diol	57-91-0	Steroid, phenolic	
17β-Estradiol	Estradiol; E2	50-28-2	Steroid, phenolic	Pharmaceutical
Estradiol benzoate	17β-Estradiol 3-benzoate	50-50-0	Steroid, phenolic	Pharmaceutical
17β-Estradiol-3-β-D-gluconate	17β-Estradiol 3-glucosiduronate; 17β-Estradiol 3-glucuronide	15270-30-1	Steroid, nonphenolic	Pharmaceutical (metabolite)
17β-Estradiol glucuronide	Estradiol 17-glucuronide; Estradiol 17β-(β-*D* -glucuronide)	1806-98-0	Steroid, nonphenolic	Pharmaceutical (metabolite)
17β-Estradiol-3-glucuronide-17-sulfate			Steroid, nonphenolic	Pharmaceutical (metabolite)
17β-Estradiol oleate		82204-99-7	Steroid, nonphenolic	
17β-Estradiol palmitate		126143-99-5	Steroid, nonphenolic	
17β-Estradiol-3-sulfate	17β-Estradiol sulfate	28814-94-0	Steroid, phenolic	
6,8-Estrapentaene-3,17β-diol	Estra-1,3,5(10),6,8-pentaene-3,17β-diol; β-Dihydroequilenin	1423-97-8	Conjugated estrogen; Steroid, phenolic	Pharmaceutical
6-Estratetraene-3,17β-diol	Estra-1,3,5(10),6-tetraene-3,17β-diol; δ6,7-Estradiol	7291-41-0	Steroid, phenolic	
7-Estratetraene-3,17β-diol	Estra-1,3,5(10),7-tetraene-3,17β-diol; β-Dihydroequilin	3563-27-7	Conjugated estrogen; Steroid, phenolic	Pharmaceutical
9-Estratetraene-3,17β-diol		791-69-5	Steroid, phenolic	
Estratriene-1,17β-diol	Estra-1,3,5(10)-triene-1,17β-diol; 1-Hydroxyestra-1,3,5(10-trien-17β-ol	126654-96-4	Steroid, phenolic	

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
Estratriene-2,17β-diol	Estra-1,3,5(10)-triene-2-17β-diol; 2,17β-Estradiol; 2-Hydroxy-3-deoxyestradiol (6CI)	2259-89-4	Steroid, phenolic	
Estratriene-3,16α-diol		1090-04-6	Steroid, phenolic	
Estratriene-3,17α-diol			Steroid, phenolic	
Estratriene-4,17β-diol	4-Hydroxyestra-1,3,5(10)-trien-17β-ol	17592-89-1	Steroid, phenolic	
Estratriene-3,11α,17β-triol	11α-Hydroxyestradiol	1464-61-5	Steroid, phenolic	
Estratriene-3,11β,17β-triol	11β-Hydroxyestradiol	5444-22-4	Steroid, phenolic	
Estratriene-3,6α,17β-triol	6α-Hydroxyestradiol	1229-24-9	Steroid, phenolic	
Estratriene-3,6β,17β-triol	Estra-1,3,5(10)-triene-3,6,17-triol; 6β-Hydroxy-17β-estradiol; 6β-Hydroxyestradiol	3583-03-7	Steroid, phenolic	
Estratriene-3,7α,17β-triol	Estra-1,3,5(10)-triene-3-7α,17β-triol; 7α-Hydroxy-17β-estradiol; 7α-Hydroxyestradiol	3398-11-6	Steroid, phenolic	
Estratriene-3,7β,17β-triol	Estra-1,3,5(10)-triene-3,7β,17β-triol; 7β-Hydroxyestradiol	2487-46-9	Steroid, phenolic	
Estratrien-17β-ol	17β-Hydroxyestra-1,3,5(10)-triene	2529-64-8	Steroid, phenolic	
Estratrien-3-ol	17-Deoxoestrone; 17-Deoxyestradiol; 3-Hydroxyestra-1,3,5(10)-triene	53-63-4	Steroid, phenolic	
Estriol	Estratriene-3,16α,17β-triol; Estratriol; 16α-Hydroxyestradiol; E3	50-27-1	Steroid, phenolic	Pharmaceutical
Estrone	Estratriene-3-ol-17-one; E1	53-16-7	Steroid, phenolic	Pharmaceutical
Ethanol		64-17-5	Alcohol	
17α-Ethinyl estradiol	19-Norpregna-1,3,5(10)-trien-20-yne-3,17-diol, (17α)-; 17α-Ethinyl estradiol	57-63-6	Steroid, phenolic	Pharmaceutical
S-Ethyl dipropylthiocarbamate	EPTC	759-94-4	Thiocarbamate	Pesticide
2-Ethylhexyl isodecyl phthalate	EHIDP	53272-22-3	Phthalate	
Ethylhexyl salicylate		118-60-5	Salicylic acid	UV light absorber (sunscreens)
Ethylparaben		120-47-8	Paraben	Preservative (pharmaceuticals)
4-Ethylphenol		123-07-9	Phenol	Flavor
Ethyl vanillin		121-32-4	Benzaldehyde	Flavor; Chemical intermediate; Perfumes
Eugenol	Phenol, 4-allyl-2-methoxy	97-53-0	Alkoxyphenol	Pharmaceutical; Cosmetic (fragrance); Chemical intermediate
Famphur		52-85-7	Organothiophosphate	Pharmaceutical (veterinary)
Fenarimol	Rubigan 12 RC	60168-88-9	Pyrimidine	Pesticide
Fenbuconazole	RH-7592; 4-(4-Chlorophenyl)-2-phenyl-2-(1H,1,2,4-triazol-1-ylmethyl)butyronitrile	14369-43-6	Nitrile; Azole	Pesticide
Fenvalerate	Pydrin; (+)-alpha-Cyano-3-phenoxybenzyl-(+)-alpha-(4-chlorophenyl)isovalerate	51630-58-1	Organochlorine; Diphenyl ether	Pesticide
Flavone		525-82-6	Flavone	Natural product
Fluazifop-butyl		69806-50-4	Pyridine	Pesticide

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
Fluoranthene		206-44-0	Polycyclic aromatic hydrocarbon	
Fluorene		86-73-7	Polycyclic aromatic hydrocarbon	Dyes
Flutamide	2-Methyl-*N* -[4-nitro-3-(trifluoro methyl)phenyl]propanamide	13311-84-7	Anilide	Pharmaceutical
Formononetin	7-Hydroxy-4'-methoxyisoflavone	485-72-3	Isoflavone	Pharmaceutical; Natural product
Gallic acid		149-91-7	Hydroxybenzoic acid	Chemical intermediate
Genistein	4,5,7-Trihydroxyisoflavanone	446-72-0	Isoflavone	Pharmaceutical; Natural product
5-OMe-Genistein			Isoflavone	
7-OMe-Genistein			Isoflavone	
Genistin		529-59-9	Isoflavone	
Gibberellic acid		77-06-5	Lactone	Plant growth regulator; Food additive
Glabridin		59870-68-7	Phenol	Natural product
Glycitein		40957-83-3	Isoflavone	
Glycitin			Isoflavone	
Glyphosate		1071-83-6	Organophosphate	Pesticide
HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid	7365-45-9	Alkyl sulfonate	Buffer
Heptachlor		76-44-8	Organochlorine; Chlorinated cyclodiene	Pesticide
2,2',3,4,4',5,5'-Heptachlorobiphenyl	PCB 180	35065-29-3	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	
4-*n* -Heptylphenol		1987-50-4	Alkylphenol	
4-*tert* -Heptylphenol			Alkylphenol	
Hexachlorobenzene		118-74-1	Organochlorine	Chemical intermediate; Pesticide; Plasticizer
2,2',3,3',5,5'-Hexachlorobiphenyl	PCB 133	35694-04-3	Polychlorinated biphenyl	Dielectric fluid
2,2',3,3',6,6'-Hexachlorobiphenyl	PCB 136	38411-22-2	Polychlorinated biphenyl	Dielectric fluid
2,2',3,4,4',5'-Hexachlorobiphenyl	PCB 138	35065-28-2	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
2,2',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	
2',3,3',4',5,5'-Hexachloro-4-biphenylol		158076-63-2	Polychlorinated biphenyl	
β-Hexachlorocyclohexane		319-85-7	Organochlorine	Pesticide
1,2,4,6,7,8-Hexahydro-4,6,6,7,8-hexamethylcyclopenta-*y* -2-benzopyrene	HHCB		Polycyclic aromatic hydrocarbon	

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
Hexazinone	Velpar	51235-04-2	Triazine	Pesticide
Hexestrol		84-16-2	Bisphenol	Pharmaceutical
Hexyl decyl phthalate	HDP		Phthalate	
Hexyl 2-ethylhexyl phthalate	HEHP		Phthalate	
4-Hexyloxyphenol	4-*n* -Hexyloxyphenol	18979-55-0	Alkylphenol	
4-*tert* -Hexylphenol		2446-69-7	Alkylphenol	
Hexyl salicylate		6259-76-3	Salicylic acid	
Homosalate	3,3,5-Trimethylcyclohexyl salicylate; Eusolex HMS	118-56-9	Salicylic acid	UV light absorber (suncreens)
3-Hydroxybenzo[β]naphtho[2,3-*d*]thiophene			Thiophene	
2-Hydroxybenzo[*c*]phenanthrene	Benzo[*c*]phenanthren-2-ol (9CI)	22717-94-8	Polycyclic aromatic hydrocarbon	
3-Hydroxybenzo[β]phenanthro[2,3-*d*]thiophene			Thiophene	
(+-)-*trans* -7,8-Hydroxybenzo[*a*]pyrene		61443-57-0	Polycyclic aromatic hydrocarbon	
1-Hydroxybenzo[*a*]pyrene	Benzo[*a*]pyren-1-ol	13345-23-8	Polycyclic aromatic hydrocarbon	
3-Hydroxybenzo[*a*]pyrene	Benzo[*a*]pyren-3-ol	13345-21-6	Polycyclic aromatic hydrocarbon	
7-Hydroxybenzo[*a*]pyrene	Benzo[*a*]pyren-7-ol	37994-82-4	Polycyclic aromatic hydrocarbon	
9-Hydroxybenzo[*a*]pyrene		17573-21-6	Polycyclic aromatic hydrocarbon	
***trans* -9,10-Hydroxybenzo[*a*]pyrene**			Polycyclic aromatic hydrocarbon	
3-Hydroxybisphenol A			Bisphenol	
1-Hydroxychlordene	Chlordene hydroxide	2597-11-7	Polycyclic aromatic hydrocarbon	
2-Hydroxychrysene	2-Chrysenol	65945-06-4	Polycyclic aromatic hydrocarbon	
6-Hydroxychrysene	6-Chrysenol	37515-51-8	Polycyclic aromatic hydrocarbon	
7-Hydroxycoumarin		93-35-6	Coumarin	Pharmaceutical metabolite
2-Hydroxyestradiol		362-05-0	Steroid, phenolic	
4-Hydroxyestradiol	4-Hydroxy-17β-estradiol	5976-61-4	Steroid, phenolic	
16α-Hydroxyestriol			Steroid, phenolic	
2-Hydroxyestriol		1232-80-0	Steroid, phenolic	
2-Hydroxyestrone		362-06-1	Steroid, phenolic	Pharmaceutical
4-Hydroxyestrone		3131-23-5	Steroid, phenolic	
11β-Hydroxyestrone		6803-21-0	Steroid, phenolic	
16-Hydroxyestrone	16α-Hydroxyestrone	18186-49-7	Steroid, phenolic	
N-β-Hydroxyethyl oleyl imidazoline			Imidazole	
2-Hydroxy-5-methylchrysene			Polycyclic aromatic hydrocarbon	

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
8-Hydroxy-5-methylchrysene			Polycyclic aromatic hydrocarbon	
***N* -(4-Hydroxyphenyl)stearamide**	Suconox-18	103-99-1	Phenol; Stearamide	
4-Hydroxyprogesterone		650-66-8	Steroid, nonphenolic	Pharmaceutical
1-Hydroxypyrene	1-Pyrenol	5315-79-7	Polycyclic aromatic hydrocarbon	
8-Hydroxyquinoline	Oxyquinoline	148-24-3	Quinoline; Phenol	Pharmaceutical; Chemical intermediate; Antiseptic; Reagent (analytical); Chelating agent
4-Hydroxytamoxifen	Hydroxytamoxifen	68047-06-3	Triphenylethylene	Pharmaceutical
6-Hydroxytetralin	5,6,7,8-Tetrahydro-2-naphthol	1125-78-6	Polycyclic aromatic hydrocarbon; Phenol	
Hydroxytoremifene		110503-62-3	Stilbene; Triphenylethylene	
ICI 164,384		98007-99-9	Steroid, phenolic	Pharmaceutical
ICI 182,780		129453-61-8	Steroid, phenolic	Pharmaceutical
Imazalil	Enilconazole	35554-44-0	Imidazole	Pesticide
Indanestrol		71855-45-3	Indane	
Indanestrol-A			Indane	
Indanestrol-B			Indane	
Indeno[*1,2,3-cd*]pyrene		193-39-5	Polycyclic aromatic hydrocarbon	
2-Iodoestratrien-17β-ol	Estra-1,3,5(10)-trien-17-ol, 2-iodo-, (17β)- (9CI)	107900-35-6	Steroid, phenolic	
3-Iodoestratrien-17β-ol	Estra-1,3,5(10)-trien-17β-ol, 3-iodo-	38605-46-8	Steroid, phenolic	
4-Iodoestratrien-17β-ol	Estra-1,3,5(10)-trien-17-ol, 4-iodo-, (17β)- (9CI)	107900-36-7	Steroid, phenolic	
Ipriflavone	7-Isopropylisoflavone	35212-22-7	Isoflavone	Pharmaceutical
Iprodion	Glycophen anphor	36734-19-7	Imidazole	Pesticide
Irganox 1640			Undetermined	
Irisolidone			Isoflavone	
Irisolidone-7-*O* -beta-*D* -glucoside			Isoflavone	
Isodecyl tridecyl phthalate	1,2-Benzenedicarboxylic acid, isodecyl tridecyl ester (9CI)	61886-60-0	Phthalate	
Isodrin		465-73-6	Organochlorine	
Isoeugenol		97-54-1	Alkoxyphenol	Natural product; Fragrance; Chemical intermediate (flavor and food additive)
Isohexylbenzyl phthalate	1,2-Benzenedicarboxylic acid, 4-methylpentyl penylmethyl ester (9CI)	1242-92-8	Phthalate	
Isoliquiritigenin	2',4',4-Trihydroxychalcone	961-29-5	Chalcone	Pharmaceutical; Natural product (phytoestrogen)
4-Isopentylphenol	Phenol,4-(3-methylbutyl)-	1805-61-4	Alkylphenol	
Isorhapontigenin	1,3-Benzenediol, 5-[(1E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]- (9CI); 3,4',5-Stilbenetriol, 3'-methoxy-	32507-66-7	Stilbene	Natural product

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
Isorhapontin	3,4',5-Trihydroxy-3'-methoxystilbene 3-*O* -β-*D* -glucopyranoside	32727-29-0	Stilbene	Natural product
Kaempferide	4-Methoxy-3,5,7-trihydroxyflavone	491-54-3	Flavone	
Kaempferol	3,4',5,7-Tetrahydroxyflavone	520-18-3	Flavone	Natural product
Kepone	1,1a,3,3a,4,5,5a,5b,6-Decachlorooctahydro-1,3,4-methano-2H-cyclobuta[*cd*]pentalen-2-one; Chlordecone	143-50-0	Organochlorine	Pesticide
6-Ketoestradiol	6-Ketoestratriene-3,17β-diol	571-92-6	Steroid, phenolic	
7-Ketoestratriene-3,17β-diol	Estra-1,3,5(10)-trien-7-one, 3,17-dihydroxy-, (17β)- (9CI); 3,17β-Dihyroxyestra-1,3,5(10)-trien-7-one	3398-12-7	Steroid, phenolic	
11-Ketoestratriene-3,17β-diol	Estra-1,3,5(10)-trien-11-one, 3,17-dihydroxy-, (17β)- (9CI)	571-65-3	Steroid, phenolic	
11-Ketotestosterone		564-35-2	Steroid, nonphenolic	
Levonorogestrel		797-63-7	Steroid, nonphenolic	Pharmaceutical
Lindane	γ-Hexachlorocyclohexane	58-89-9	Organochlorine	Pesticide; Pharmaceutical
Linuron		330-55-2	Urea	Pesticide
Luteolin	3',4',5,7-Tetrahydroxyflavone	491-70-3	Flavone	Pharmaceutical
Malathion	Carbafos	121-75-5	Organophosphate	Pesticide
Maneb	Manganese ethylene-1,2-bis-dithiocarbamate	12427-38-2	Carbamate	Pesticide
Melengesterol acetate		2919-66-6	Steroid, nonphenolic	Pharmaceutical
Menthyl salicylate	5-Methyl-2-(1-methylethyl)cyclohexyl 2-hydroxybenzoate	89-46-3	Salicylic acid	Pharmaceutical additive
Mestranol		72-33-3	Steroid, nonphenolic	Pharmaceutical
Methomyl		16752-77-5	Carbamate	Pesticide
Methoprene	Altosid	40596-69-8	Carboxylic acid	Fertilizer; Growth regulator
Methoxybisphenol A			Bisphenol	
Methoxychlor	(1,1,1-Trichloro-2,2-bis(*p* -methoxyphenyl)-ethane	72-43-5	Organochlorine	Pesticide
***o,p'* -Methoxychlor**		30667-99-3	Organochlorine	Pesticide
2-Methoxyestrone		362-08-3	Steroid, phenolic	
4-Methoxyestrone	1, 3, 5(10)-Estratrien-3, 4-diol-17-one 4-methyl ether		Steroid, phenolic	
3-(4-Methylbenzylidene)camphor	Eusolex 6300	36861-47-9	Undetermined	
4,4'-Methylenebis(2,6-di-*tert* -butylphenol)		118-82-1	Bisphenol	
2,2'-Methylenebis(4-methyl-6-*tert* -butylphenol)		119-47-1	Bisphenol	
2-Methylnaphthalene		91-57-6	Polycyclic aromatic hydrocarbon	Chemical intermediate
4-Methyl-2-nonylphenol			Alkylphenol	
Methylparaben		99-76-3	Paraben	Preservative (foods and cosmetics)

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
Methyl parathion		298-00-0	Organothiophosphate	Pesticide
1-Methyl-1-phenylindan		79034-12-1	Undetermined	
Methyl salicylate		119-36-8	Salicylic acid	
Methyltestosterone		58-18-4	Steroid, nonphenolic	Pharmaceutical
Methyltrienolone	R1881; 17-β-Hydroxy-17-methylestra-4,9,11-trien-3-one	965-93-5	Steroid, nonphenolic	Pharmaceutical
Metiram	Poly(ethylenethiuram disulfide)	9006-42-2	Carbamate	Pesticide
Metolachlor	Humextra	51218-45-2	Acetamide	Pesticide
Mifepristone	RU 486	84371-65-3	Steroid, nonphenolic	Pharmaceutical
Mirex	1,1a,2,2,3,3a,4,5,5,5a,5b,6-Dodecachlorooctahydro-1,3,4-metheno-1H-cyclobutra(cd)pentalene	2385-85-5	Organochlorine	Pesticide; Fire retardant for plastics, rubber, paint
3-Monobromobisphenol A	MBBPA		Bisphenol	
Monohydroxy-DDE			Organochlorine	Pesticide metabolite
Monohydroxymethoxychlor		28463-03-8	Organochlorine	Pesticide metabolite
Moxestrol	11β-Methoxyethinylestradiol; R 2858	34816-55-2	Steroid, phenolic	Pharmaceutical
Nafoxidine	Pyrrolidine, 1-(2-(4-(3,4-dihydro-6-methoxy-2-phenyl-1-naphthalenyl)phenoxy)ethyl)-	1845-11-0	Triphenylethylene; Stilbene	Pharmaceutical
Naphthalene		91-20-3	Polycyclic aromatic hydrocarbon	Chemical intermediate
α-Naphthoflavone	7,8-Benzoflavone	604-59-1	Flavone	Enzyme inhibitor
1-Naphthol	1-Hydroxynaphthalene	90-15-3	Polycyclic aromatic hydrocarbon; Phenol	Chemical intermediate
2-Naphthol	2-Hydroxynaphthalene	135-19-3	Polycyclic aromatic hydrocarbon; Phenol	Dyes; Pigments; Antioxidant (rubber, fats, oil); Pharmaceutical; Pesticide
1,2-Naphthoquinone		524-42-5	Quinone	Reagent
Naringenin	4',5,7-Trihydroxyflavanone	480-41-1	Flavanone	
Niacinamide	Nicotinamide; Vitamin B3	98-92-0	Nicotinic acid	Pharmaceutical
2-Nitroestratriene-3,17β-diol	2-Nitro-1,3,5(10)-estratriene-3,17β-diol	6298-51-7	Steroid, phenolic	
4-Nitroestratriene-3,17β-diol	4-Nitro-1,3,5(10)-estratriene-3,17β-diol; 4-Nitroestradiol	6936-94-3	Steroid, phenolic	
1-Nitroestratrien-17β-ol	Estra-1,3,5(10)-trien-17-ol, 1-nitro-, (17β)- (9CI)	194068-94-3	Steroid, phenolic	
2-Nitroestratrien-17β-ol		101772-26-3	Steroid, phenolic	
3-Nitroestratrien-17β-ol	Estra-1,3,5(10)-trien-17-ol, 3-nitro, (17β)- (9CI)	197068-95-4	Steroid, phenolic	
4-Nitroestratrien-17β-ol		101772-25-2	Steroid, phenolic	
4-Nitrotoluene	*p* -Nitrotoluene	99-99-0	Aromatic hydrocarbon	Chemical intermediate
***cis* -Nonachlor**		5103-73-1	Organochlorine; Chlorinated bridged cycloalkene	Pesticide
***trans* -Nonachlor**		39765-80-5	Organochlorine; Chlorinated bridged cycloalkene	Pesticide
Nonylphenol		84852-15-3	Alkylphenol	

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
***n* -Nonylphenol**	nonylphenol n	25154-52-3	Alkylphenol	Chemical intermediate
***o* -Nonylphenol**	*o* -Nonylphenol	136-83-4	Alkylphenol	
***p* -Nonylphenol**	4-Nonylphenol; 4-*n* -Nonylphenol	104-40-5	Alkylphenol	Chemical intermediate
***p* -Nonylphenol benzoate ester**			Alkylphenol	
Nonylphenol diethoxylate			Alkylphenyl ether	Surfactant
4-Nonylphenol diethoxylate			Alkylphenyl ether	Surfactant
Nonylphenol heptaethoxylate			Alkylphenyl ether	Surfactant
4-Nonylphenoxycarboxylic acid			Alkylphenyl ether	
1-*O* -(Nonylphenyl)-α,β-*D* -glucopyranosiduric acid			Alkylphenyl ether	
Nordihydroguaiaretic acid		500-38-9	Bisphenol	Pharmaceutical; Natural product
Norethindrone		68-22-4	Steroid, nonphenolic	Pharmaceutical
Norgestrel		6533-00-2	Steroid, nonphenolic	Pharmaceutical
19-Nortestosterone		434-22-0	Steroid, nonphenolic	Pharmaceutical
Octachlorostyrene	Trichlorovinylpentachlorobenzene	29082-74-4	Organochlorine	Chemical intermediate
Octadecyl-3-(3',5'-di-*tert* -butyl-4-hydroxyphenyl)propionate	Irganox 1076	2082-79-3	Cresol	Antioxidant
Octrizole		3147-75-9	Triazole; Phenol	UV light absorber
Octyldimethyl-*p* -aminobenzoic acid	2-Ethylhexyl-4-dimethyl aminobenzoate; Eusolex 6007	21245-02-3	Aminobenzoic acid	Pharmaceutical
***n* -Octyl gallate**		1034-01-1	Hydroxybenzoic acid	Preservative
Octyl isodecyl phthalate	OIDP	1330-96-7	Phthalate	Plasticizer
Octyl methoxycinnamate	2-Ethylhexyl-4-methoxycinnamate; Eusolex 2292	5466-77-3	Carboxylic acid; Cinnamate	Pharmaceutical
Octylphenol	(1,1,3,3-Tetramethylbutyl)phenol	27193-28-8	Alkylphenol	
4*n* -Octylphenol	4-*n* -Octylphenol; *p* -Octylphenol	1806-26-4	Alkylphenol	Chemical intermediate
4-*tert* -Octylphenol	4-(1,1,3,3-Tetramethylbutyl)phenol; *p-tert* -Octylphenol	140-66-9	Alkylphenol	Chemical intermediate
5-Octylphenol			Alkylphenol	
1-*O* -(Octylphenyl)-α,β-*D* -glucopyranosiduronic acid			Alkylphenol; Glucuronic acid conjugate	
Org 2058	16alpha-Ethyl-21-hydroxy-19-nor-4-pregnene-3,20-dione	24320-06-7	Steroid, nonphenolic	
Oxamyl		23135-22-0	Carbamate	Pesticide
Paraquat		4685-14-7	Pyridine	Pesticide
Parathion		56-38-2	Organothiophosphate	Pesticide
2,2',3,5',6-Pentachlorobiphenyl	PCB 95	38379-99-6	Polychlorinated biphenyl	Dielectric fluid
2,2',4,6,6'-Pentachlorobiphenyl	PCB 104	56558-16-8	Polychlorinated biphenyl	Dielectric fluid
2,3,3',4,4'-Pentachlorobiphenyl	PCB 105	32598-14-4	Polychlorinated biphenyl	Dielectric fluid
2,3,3',4,5-Pentachlorobiphenyl	PCB 106	70424-69-0	Polychlorinated biphenyl	Dielectric fluid
2,3,4,5,6-Pentachlorobiphenyl	PCB 116	18259-05-7	Polychlorinated biphenyl	Dielectric fluid

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
3,3',4,4',5-Pentachlorobiphenyl	PCB 126	57465-28-8	Polychlorinated biphenyl	Dielectric fluid
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	4-Hydroxy-2',3,3',4',5-pentachlorobiphenyl	149589-55-9	Polychlorinated biphenyl	
2',3,3',4',5'-Pentachloro-4-biphenylol	4-Hydroxy-2',3,3',4',5'-pentachlorobiphenyl	192190-09-3	Polychlorinated biphenyl	
2',3,3',4',6'-Pentachloro-4-biphenylol	4-Hydroxy-2',3,3',4',6'-pentachlorobiphenyl	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-2-biphenylol		67651-36-9	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	
Pentachlorophenol		87-86-5	Organochlorine	Pesticide
4-*n* -Pentylphenol	4-*n* -Amylphenol	14938-35-3	Alkylphenol	Chemical intermediate
Permethrin	(3-Phenoxyphenyl)methyl (+-) *cis,trans* -3-(2,2-dichloroethenyl)-2,2-dimethyl cyclopropanecarboxylate	52645-53-1	Pyrethrin	Pesticide
Perylene		198-55-0	Polycyclic aromatic hydrocarbon	Fluorescent probe; Manufacture of semi-conductors
Phenanthrene		85-01-8	Polycyclic aromatic hydrocarbon	Chemical intermediate
Phenobarbital, sodium salt		57-30-7	Pyrimidine	Pharmaceutical
Phenol		108-95-2	Phenol	Chemical intermediate; Pharmaceutical (antiseptic); Disinfectant
4-Phenoxyphenol	4-Hydroxydiphenyl ether	831-82-3	Phenol; Ether	
1-Phenyl-3,5-*p* -hydroxyphenyl-4-ethylpyrazole			Pyrazole	
2-Phenyl indole		948-65-2	Indole	
Phenyl salicylate	Salol	118-55-8	Salicylic acid	Pharmaceutical
4-Phenyl toluene	4-Methylbiphenyl	644-08-6	Biphenyl	
Phloretin	(4-Hydroxyphenyl)-2,4,6-trihydroxypropiophenon	60-82-2	Flavone	
Piceatannol	3,5,3',4'-Tetrahydroxystilbene	10083-24-6	Stilbene	Pharmaceutical
Picloram		1918-02-1	Undetermined	Pesticide; Plant growth regulator
Pinosylvin	3,5-Dihydroxystilbene	22139-77-1	Stilbene	

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
Podocarpic acid		5947-49-9	Polycyclic aromatic hydrocarbon	
Polybrominated diphenyl ether 15	PBDE 15; 4,4'-Dibromodiphenyl ether		Polybrominated diphenyl ether	Flame retardant additive
Polybrominated diphenyl ether 28	PBDE 28; 2,4,4'-Tribromodiphenyl ether		Polybrominated diphenyl ether	Flame retardant additive
Polybrominated diphenyl ether 30	PBDE 30; 2,4,6-Tribromodiphenyl ether		Polybrominated diphenyl ether	Flame retardant additive
Polybrominated diphenyl ether 32	PBDE 32; 2,4',6-Tribromodiphenyl ether		Polybrominated diphenyl ether	Flame retardant additive
Polybrominated diphenyl ether 47	PBDE 47; 2,2',4,4'-Tetrabromodiphenyl ether		Polybrominated diphenyl ether	Flame retardant additive
Polybrominated diphenyl ether 51	PBDE 51; 2,2',4,6'-Tetrabromodiphenyl ether		Polybrominated diphenyl ether	Flame retardant additive
Polybrominated diphenyl ether 71	PBDE 71; 2,3',4',6-Pentabromodiphenyl ether	32534-81-9	Polybrominated diphenyl ether	Flame retardant additive
Polybrominated diphenyl ether 75	PBDE 75; 2,4,4',6-Tetrabromodiphenyl ether		Polybrominated diphenyl ether	Flame retardant additive
Polybrominated diphenyl ether 77	PBDE 77; 3,3',4,4'-Tetrabromodiphenyl ether		Polybrominated diphenyl ether	Flame retardant additive
Polybrominated diphenyl ether 85	PBDE 85; 2,2',3,4,4'-Pentabromodiphenyl ether		Polybrominated diphenyl ether	Flame retardant additive
Polybrominated diphenyl ether 99	PBDE 99; 2,2',4,4',5-Pentabromodiphenyl ether		Polybrominated diphenyl ether	Flame retardant additive
Polybrominated diphenyl ether 100	PBDE 100; 2,2',4,4',6-Pentabromodiphenyl ether		Polybrominated diphenyl ether	Flame retardant additive
Polybrominated diphenyl ether 119	PBDE 119; 2,3,4,4',6-Pentabromodiphenyl ether		Polybrominated diphenyl ether	Flame retardant additive
Polybrominated diphenyl ether 138	PBDE 138; 2,2',3,4,4',5'-Heptabromodiphenyl ether		Polybrominated diphenyl ether	Flame retardant additive
Polybrominated diphenyl ether 153	PBDE 153; 2,2',4,4',5,5-Hexabromodiphenyl ether		Polybrominated diphenyl ether	Flame retardant additive
Polybrominated diphenyl ether 166	PBDE 166; 2,3,4,4',5,6-Hexabromodiphenyl ether		Polybrominated diphenyl ether	Flame retardant additive
Polybrominated diphenyl ether 190	PBDE 190; 2,3,3',4,4',5,6-Heptabromodiphenyl ether		Polybrominated diphenyl ether	Flame retardant additive
Prochloraz	N-propyl-N-(2-(2,4,6-trichlorophenoxy)ethyl)-1H-imidazole	67747-09-5	Imidazole	Pesticide
Procymidone	(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide	32809-16-8	Organochlorine; Imide	Pesticide
Progesterone	Pregn-4-ene-3,20-dione	57-83-0	Steroid, nonphenolic	Pharmaceutical
17α-Progesterone		2000-66-0	Steroid, nonphenolic	Pharmaceutical
17α,20β-Progesterone			Steroid, nonphenolic	
Promegestone	R5020; 17,21-Dimethyl-19-nor-4,9-pregnadiene-3,20-dione	34184-77-5	Steroid, nonphenolic	Pharmaceutical
Propazine	2-Chloro-4,6-bis(isopropylamino)-s-triazine	139-40-2	Triazine	Pesticide
Propham		122-42-9	Carbamate	Pesticide
Propioconazole		60207-90-1	Triazole; Organochlorine	Pesticide
Propoxylated 4-nonylphenol			Alkylphenyl ether	Surfactant
***n* -Propyl gallate**		121-79-9	Benzoic acid; Phenol	Preservative (foods)

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
Propylparaben		94-13-3	Paraben	Pharmaceutical; Preservative (foods)
4-Propylphenol		645-56-7	Alkylphenol	
Propylthiourea		927-67-3	Urea	Pesticide
4-Propyl-1,3,5-tris(4-hydroxyphenyl)pyrazole			Pyrazole	
Propyzamide	Pronamide	23950-58-5	Benzamide	Pesticide
Pseudodiethylstilbestrol	Pseudo-DES	39011-86-4	Stilbene; Bisphenol	
Pseudodiethylstilbestrol-e	Phenol, 4,4'-(1-ethyl-2-ethylidene-1,2-ethanediyl)bis-, (E)- (9CI); (E)-ψ-Diethylstilbestrol	81493-97-2	Stilbene; Bisphenol	DES metabolite
Pseudodiethylstilbestrol-z	Phenol, 4,4'-(1-ethyl-2-ethylidene-1,2-ethanediyl)bis-, (Z)- (9CI)	85546-05-0	Stilbene; Bisphenol	DES metabolite
Purpurogallin	2,3,4,6-Tetrahydroxy-5H-benzocyclohepten-5-one	569-77-7	Polycyclic aromatic hydrocarbon	Pharmaceutical
Pyrene		129-00-0	Polycyclic aromatic hydrocarbon	Dyes
Pyrethrins	Pyrethrum	8003-34-7	Pyrethrin	Pesticide
Quercetin	3,3,'4',5,7-Pentahydroxyflavone	117-39-5	Flavone	Natural product (plant)
Quintozen	Pentachloronitrobenzene	82-68-8	Organochlorine	Pesticide; Chemical intermediate
Raloxifene		84449-90-1	Phenol; Nitrogen heterocycle; Sulfur heterocycle	Pharmaceutical
Resorcinol monobenzoate		136-36-7	Resorcinol	UV light absorber (plastics)
Resveratrol	3,4',5-Stilbenetriol	501-36-0	Stilbene	Pharmaceutical
***trans* -Retinoic acid**	Vitamin A acid	302-79-4	Retinoid	Pharmaceutical
Rimsulfuron		122931-48-0	Sulfonylurea	Pesticide
Rotenone		83-79-4	Pyrethrin	Pesticide
Salicyclic acid		69-72-7	Salicylic acid	Pharmaceutical; Preservative (foods); Chemical intermediate
Sarsasapogenin	Spirostan-3β-ol	126-19-2	Steroid, nonphenolic	
Silvex	Propanoic acid, 2-(2,4,5-trichlorophenoxy)-	93-72-1	Organochlorine	Pesticide
Simazine	2-Chloro-4,6-bis(ethylamino)-S-triazine; 1,3,5-Triazine-2,4-diamine, 6-chloro-*N*,*N'* -diethyl-	122-34-9	Organochlorine; Triazine	Pesticide
Sissotrin	7-β-*D* -Glucosyl-5,7-dihydroxy-4'-methoxyisoflavone; Biochanin A 7-β-*D* -glucopyranoside; Biochanin A 7-*O* -β-glucoside	5928-26-7	Isoflavone	Natural product
β-Sitostanol		83-45-4	Steroid, nonphenolic	Pharmaceutical
β-Sitosterol		83-46-5	Steroid, nonphenolic	Natural product
Sodium lignosulfonate		8061-51-6	Undetermined	Emulsion stabilizer; Chelating agent
Sodium *n* -nonyl sulfate		1072-15-7	Hydrocarbon	
Sodium *n* -octyl sulfate		142-31-4	Hydrocarbon	Pharmaceutical
Spruce Lignan		9005-53-2	Undetermined	
***trans* -Stilbene**	*trans* -1,2-Diphenylethylene	103-30-0	Stilbene	Chemical intermediate; Scintillator

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
Styrene	Vinylbenzene	100-42-5	Aromatic hydrocarbon	Chemical intermediate; Monomer; Cross-linking agent; Plastics; Coatings; Flavor
Sumithrin	Phenothrin	26002-80-2	Pyrethrin	Pesticide
Tamoxifen	ICI 47699	10540-29-1	Stilbene	Pharmaceutical
***cis* -Tamoxifen**	Tamoxifen (E)	13002-65-8	Stilbene	
Tamoxifen citrate	Kessar; Noltam; Nolvadex	54965-24-1	Stilbene	Pharmaceutical
Tamoxifen metabolite E		68684-63-9	Stilbene	Pharmaceutical metabolite
Tectoridin	4*H* -1-Benzopyran-4-one, 7- (β-*D* -glucopyranosyloxy)-5-hydroxy-3-(4-hydroxyphenyl)-6-methoxy- (9CI)	611-40-5	Isoflavone	Natural product
Tectorigenin	4',5,7-Trihydroxy-6-methoxyisoflavone	548-77-6	Isoflavone	Pharmaceutical
Terbacil		5902-51-2	Urea	Pesticide
Testosterone	Androst-4-en-3-one, 17-hydroxy-, (17β)-	58-22-0	Steroid, nonphenolic	Pharmaceutical
Tetrabromobisphenol A		79-94-7	Bisphenol	
2,2',4,5-Tetrachlorobiphenyl	PCB 48	70362-47-9	Polychlorinated biphenyl	Dielectric fluid
2,2',5,5'-Tetrachlorobiphenyl	PCB 52	35693-99-3	Polychlorinated biphenyl	Dielectric fluid
2,2',6,6'-Tetrachlorobiphenyl	PCB 54	15968-05-5	Polychlorinated biphenyl	Dielectric fluid
2,3',4,4'-Tetrachlorobiphenyl	PCB 66	32598-10-0	Polychlorinated biphenyl	Dielectric fluid
2,3,4,4'-Tetrachlorobiphenyl	PCB 60	33025-41-1	Polychlorinated biphenyl	Dielectric fluid
2,3,4,5-Tetrachlorobiphenyl	PCB 61	33284-53-6	Polychlorinated biphenyl	Dielectric fluid
2,3,5,6-Tetrachlorobiphenyl	PCB 65	33284-54-7	Polychlorinated biphenyl	Dielectric fluid
2,4,4',6-Tetrachlorobiphenyl	PCB 75	32598-12-2	Polychlorinated biphenyl	Dielectric fluid
3,3',4,4'-Tetrachlorobiphenyl	PCB 77	32598-13-3	Polychlorinated biphenyl	Dielectric fluid
3,3',5,5'-Tetrachlorobiphenyl	PCB 80	33284-52-5	Polychlorinated biphenyl	Dielectric fluid
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	3-Hydroxy-2',3',4',5'-tetrachlorobiphenyl	67651-37-0	Polychlorinated biphenyl	
2',3',4',5'-Tetrachloro-4-biphenylol	4-Hydroxy-2',3',4',5'-tetrachlorobiphenyl	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	
Tetrachloroethylene	Perchloroethylene	127-18-4	Chlorinated hydrocarbon	Chemical intermediate; Solvent
Tetrachlorvinphos	Stirofos	22248-79-9	Organophosphate	Pesticide
Tetradifon	*p* -Chlorophenyl 2,4,5-trichlorophenyl sulfone	116-29-0	Chlorinated hydrocarbon	Pesticide
2',4',4,6'-Tetrahydroxychalcone		73692-50-9	Chalcone	
Thiram	Tetramethylthiuram disulfide	137-26-8	Dithiocarbamate	Pesticide

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
Thymol	I-Hydroxy-5 methyl-2-isopropylbenzene	89-83-8	Alkylphenol	Flavor; Preservative; Pharmaceutical; Fragrance; Disinfectant (oral hygiene products)
Toxaphene		8001-35-2	Organochlorine	Pesticide
Tralomethrin		66841-25-6	Pyrethrin	Pesticide
Trans-**3b**			Undetermined	
Trans-**3c**			Undetermined	
Transforming Growth Factor α (TGF–α)	TGF-α		Polypeptide	Natural product
β-Trenbolone	17-beta-Hydroxyestra-4,9,11-trien-3-one	10161-33-8	Steroid, nonphenolic	Pharmaceutical
Trenbolone acetate	17-beta-(Acetyloxy)estra-4,9,11-trien-3-one	10161-34-9	Steroid, nonphenolic	Pharmaceutical
Triadimefon	1-(4-Chlorophenoxy)-3,3-dimethyl-1(1,2,4-triazol-1-y) butanone	43121-43-3	Triazole	Pesticide
Triadimenol	Triaphol	55219-65-3	Triazole	Pesticide
3,3',5-Tribromobisphenol A	triBBPA	6386-73-8	Bisphenol	
4-(2,4,6-Tribromophenoxy)phenol			Phenol; Phenyl ether	
2,2',3-Trichlorobiphenyl	PCB 16	38444-78-9	Polychlorinated biphenyl	Dielectric fluid
2,2',6-Trichlorobiphenyl	PCB 19	38444-73-4	Polychlorinated biphenyl	Dielectric fluid
2,3,4-Trichlorobiphenyl	PCB 21	55702-46-0	Polychlorinated biphenyl	Dielectric fluid
2,3',5-Trichlorobiphenyl	PCB 26	38444-81-4	Polychlorinated biphenyl	Dielectric fluid
2,3,6-Trichlorobiphenyl	PCB 24	55702-45-9	Polychlorinated biphenyl	Dielectric fluid
2,3',6-Trichlorobiphenyl	PCB 27	38444-76-7	Polychlorinated biphenyl	Dielectric fluid
2,4,6-Trichlorobiphenyl	PCB 30	35693-92-6	Polychlorinated biphenyl	Dielectric fluid
3,4',5-Trichlorobiphenyl	PCB 39	38444-88-1	Polychlorinated biphenyl	Dielectric fluid
2,4,6-Trichloro-3',4'-biphenyldiol			Polychlorinated biphenyl	
2,2',5-Trichloro-4-biphenylol			Polychlorinated biphenyl	
2',4',6'-Trichloro-4-biphenylol		14962-28-8	Polychlorinated biphenyl	
3,3',4'-Trichloro-4-biphenylol	4-Hydroxy-3,3',4'-trichlorobiphenyl	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	93-76-5	Organochlorine	Pesticide
Triethanolamine salicylate	TEA-Salicylate	2174-16-5	Salicylic acid; Ethanolamine	Pharmaceutical
Trifluralin	Benzamine, 2,6-dinitro-*N,N* -dipropyl-4-(trifluoromethyl)	1582-09-8	Aniline	Pesticide
2,4,5-Trihydroxybutyrophenone		1421-63-2	Phenol	Preservative (foods, paraffin waxes)
Trihydroxymethoxychlor			Organochlorine	Pesticide metabolite
3,3',5'-Triiodo-1-thyronine	T3; 3,5,3'-Triiodothyronine	6893-02-3	Amino acid derivative	Pharmaceutical
Trimethoxymethoxychlor			Organochlorine	
1,3,5-Trimethyl-2,4,6-tris(3,5-di-*tert* -butyl-4-hydroxybenzyl)benzene	Ionox 330; Irganox 1330	1709-70-2	Cresol	Antioxidant (Adhesives, films, pipes, cable, molding, packaging)

Information Sorted by Substance Name

Abietic acid		514-10-3	Polycyclic hydrocarbon	Pharmaceutical
Triphenyltin chloride		639-58-7	Organometallic compound	Pesticide
1,3,5-Tris(4-*tert* -butyl-3-hydroxy-2,6,-dimethylbenzyl)isocyanurate		40601-76-1	Undetermined	
Tris-4-(chlorophenyl)methane	Tris-H	27575-78-6	Organochlorine	
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)
Vinclozolin	3-(3,5-Dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione	50471-44-8	Organochlorine	Pesticide
Vitamin E	Alpha-tocopherol	59-02-9	Benzopyranol	Pharmaceutical; Animal feed (additive); Antioxidant (shortening and oils)
***p* -Xylene**	1,4-Dimethylbenzene	106-42-3	Aromatic hydrocarbon	Solvent (manufacture of paints, dyes, coatings, rubber); Chemical intermediate (synthesis of organic chemicals); Aviation gasoline
α-Zearalanol	Zearanol	26538-44-3	Resorcyclic acid lactone	Natural product
β-Zearalanol		42422-68-4	Resorcyclic acid lactone	Natural product
Zearalanone		5975-78-0	Resorcyclic acid lactone	Natural product
α-Zearalenol		36455-72-8	Resorcyclic acid lactone	Natural product
β-Zearalenol		71030-11-0	Resorcyclic acid lactone	Natural product
Zearalenone		17924-92-4	Resorcyclic acid lactone	Natural product
Zeranol		55331-29-8	Phenol	
Zineb	Zinc ethylenebis(dithiocarbamate)	12122-67-7	Dithiocarbamate	Pesticide
Ziram		137-30-4	Dithiocarbamate	Pesticide

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Appendix D

Substances Tested in the *In Vitro* ER TA Assays

D1 Information Sorted by Chemical Name and Assay

D2 References

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Appendix D1

Substances Tested in the *In Vitro* ER TA Assays

Information Sorted by Chemical Name and Assay

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Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
MCF-7 hER(E)+CP	Abietic acid	514-10-3				pos.			Mellanen et al. (1996)
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	Acenaphthene	83-32-9	neg.				neg.	96	Tran et al. (1996)
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	Acenaphthene	83-32-9					neg.		Tran et al. (1996)
BG-1 hER(E)+Luc(S)	Acenaphthylene	208-96-8	neg.						Xenobiotic Detection Systems (2001)
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	Acenaphthylene	208-96-8	neg.				neg.	100	Tran et al. (1996)
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	Acenaphthylene	208-96-8					neg.		Tran et al. (1996)
HEK293 hERα(S)+Luc(T)	6-Acetyl-1,1,2,4,4,7-hexamethyltetraline	21145-77-7	neg.						Seinen et al. (1999)
HEK293 hERα(T)+Luc(T)	6-Acetyl-1,1,2,4,4,7-hexamethyltetraline	21145-77-7	pos.						Seinen et al. (1999)
HEK293 hERβ(S)+Luc(T)	6-Acetyl-1,1,2,4,4,7-hexamethyltetraline	21145-77-7	neg.						Seinen et al. (1999)
HEK293 hERβ(T)+Luc(T)	6-Acetyl-1,1,2,4,4,7-hexamethyltetraline	21145-77-7	neg.						Seinen et al. (1999)
CHO-K1 hERα(T)+Luc(T)†	Acrinathrin	101007-06-1	neg.						Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Acrinathrin	101007-06-1				neg.			Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Acrinathrin	101007-06-1				neg.			Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Alachlor	15972-60-8				neg.			Soto et al. (1995)
MCF-7 hER(E)+Luc(T)+β-gal(T)	Alachlor	15972-60-8		0.5					Klotz et al. (1996)
Yeast(*S.cer.* BJ2407) hER(S)+β-gal(S)	Alachlor	15972-60-8		40x (10 µM)*			neg.		Klotz et al. (1996)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Alachlor	15972-60-8	pos.	18.19					Petit et al. (1997)
Ishikawa hER(T)+Luc(T)+β-gal(T)	Aldicarb	116-06-3	neg.	0.06			pos.	12%	Klotz et al. (1997)
MCF-7 hER(E)+Luc(T)+β-gal(T)	Aldicarb	116-06-3		0.06				24%	Klotz et al. (1997)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
CHO-K1 hERα(T)+Luc(T)†	Aldosterone	52-39-1	neg.		0				Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Allenolic acid	553-39-9				pos.			Soto et al. (1995)
MCF-7 hER(E)+CP	δ-*trans* -Allethrin	584-79-2				pos.			Go et al. (1999)
MCF-7(E3) hER(E)+CP	2-Aminoestratriene-3,17β-diol	107900-30-1			0.558	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	4-Aminoestratriene-3,17β-diol	107900-31-2			0.346	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	1-Aminoestratrien-17β-ol				28.4	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	2-Aminoestratrien-17β-ol	17522-06-4			0.127	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	3-Aminoestratrien-17β-ol				1.5	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	4-Aminoestratrien-17β-ol	17522-04-2			2	pos.			Wiese et al. (1997)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Aminotriazole	61-82-5	pos.	17.78					Petit et al. (1997)
MCF-7 hER(E)+CP	4-*tert* -Amylphenol	80-46-6				pos.			Soto et al. (1995)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	4-*tert* -Amylphenol	80-46-6	pos.	0.000005					Miller et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-*tert* -Amylphenol	80-46-6	pos.	0.000004	50				Routledge and Sumpter (1997)
MCF-7(E3) hER(E)+CP	5α-Androstane-3α,17β-diol	1852-53-5			0	neg.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	5α-Androstane-3β,17β-diol	571-20-0			4.66	pos.			Wiese et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	4-Androstenediol	1156-92-9	pos.	0.023					Coldham et al. (1997)
MCF-7(E3) hER(E)+CP	5-Androstenediol	521-17-5			1.41	pos.			Wiese et al. (1997)
CHO-K1 hERα(T)+Luc(T)†	Androstenedione	63-05-8	neg.		0				Otsuka Pharmaceutical (2001)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	4-Androstenedione	63-05-8	neg.	0					Coldham et al. (1997)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	4-Androstenedione	63-05-8	neg.						Le Guevel and Pakdel (2001)
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	Anthanthrene	191-26-4	neg.				neg.	97	Tran et al. (1996)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	Anthanthrene	191-26-4					neg.		Tran et al. (1996)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Anthracene	120-12-7	neg.		0				Clemons et al. (1998)
MCF-7 hER(E)+CP(F)	Anthracene	120-12-7				neg.	neg.		Arcaro et al. (1999b)
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	Anthracene	120-12-7	neg.				neg.	97	Tran et al. (1996)
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	Anthracene	120-12-7					neg.		Tran et al. (1996)
HEK-293 hERα(T)+Luc(T)	Apigenin	520-36-5	pos.	40			pos.	68.1	Collins-Burow et al. (2000)
HEK-293 hERα(T)+Luc(T)	Apigenin	520-36-5	weak				pos.	78.3	Collins-Burow et al. (2000)
HEK293 hERα(T)+Luc(T)+β-gal(T)	Apigenin	520-36-5	pos.	50			neg.		Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	Apigenin	520-36-5	pos.	49			neg.		Kuiper et al. (1998)
HeLa hER(T)+CAT(T)	Apigenin	520-36-5	pos.	27.3					Miksicek (1993)
MCF-7 hER(E)+CP	Apigenin	520-36-5				pos.			Miksicek (1993)
MCF-7(M) hER(E)+Luc(T)+β-gal(T)	Apigenin	520-36-5	pos.	60			pos.		Collins-Burow et al. (2000)
MCF-7(M) hER(E)+CP	Apigenin	520-36-5				neg.	pos.		Collins-Burow et al. (2000)
BG-1 hER(E)+Luc(S)	Aroclor 1016	12674-11-2	pos.						Xenobiotic Detection Systems (2001)
BG-1 hER(E)+Luc(S)	Aroclor 1221	11104-28-2	pos.						Xenobiotic Detection Systems (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Aroclor 1221	11104-28-2	pos.	56.21					Petit et al. (1997)
BG-1 hER(E)+Luc(S)	Aroclor 1232	11141-16-5	pos.						Xenobiotic Detection Systems (2001)
BG-1 hER(E)+Luc(S)	Aroclor 1242	53469-21-9	pos.						Xenobiotic Detection Systems (2001)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
BG-1 hER(E)+Luc(S)	Aroclor 1248	12672-29-6	neg.						Xenobiotic Detection Systems (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Aroclor 1248	12672-29-6	pos.	37.47					Petit et al. (1997)
BG-1 hER(E)+Luc(S)	Aroclor 1254	11097-69-1	neg.						Xenobiotic Detection Systems (2001)
BG-1 hER(E)+Luc(S)	Aroclor 1260	11096-82-5	neg.						Xenobiotic Detection Systems (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Aroclor 1268	11100-14-4	pos.	61.08					Petit et al. (1997)
CHO-K1 hERα(T)+Luc(T)†	Atrazine	1912-24-9	neg.		0				Otsuka Pharmaceutical (2001)
HeLa hER(S)+Luc(S)	Atrazine	1912-24-9	neg.						Balaguer et al. (1996)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Atrazine	1912-24-9	neg.	0			neg.		Connor et al. (1996)
MCF-7 hER(E)+CP	Atrazine	1912-24-9				neg.			Soto et al. (1995)
Yeast(*S.cer* .188R1) hER(S)+β-gal(S)	Atrazine	1912-24-9	neg.				neg.		Graumann et al. (1999)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Atrazine	1912-24-9	pos.	16.51					Petit et al. (1997)
Ishikawa hER(T)+Luc(T)+β-gal(T)	Baygon	114-26-1	neg.	0.07			pos.	46%	Klotz et al. (1997)
MCF-7 hER(E)+Luc(T)+β-gal(T)	Baygon	114-26-1		0.07				30%	Klotz et al. (1997)
Ishikawa hER(T)+Luc(T)+β-gal(T)	Bendiocarb	22781-23-3	neg.	0.07			pos.	43%	Klotz et al. (1997)
MCF-7 hER(E)+CP	Bendiocarb	22781-23-3				neg.			Soto et al. (1995)
MCF-7 hER(E)+Luc(T)+β-gal(T)	Bendiocarb	22781-23-3		0.07				30%	Klotz et al. (1997)
MCF-7 hER(E)+Luc(T)+β-gal(T)	Benomyl	17804-35-2		0.04					Klotz et al. (1996)
Yeast(*S.cer.* BJ2407) hER(S)+β-gal(S)	Benomyl	17804-35-2		0 (10 µM)*			neg.		Klotz et al. (1996)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	2,3-Benzanthracene	92-24-0	neg.				neg.	101	Tran et al. (1996)
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	2,3-Benzanthracene	92-24-0					neg.		Tran et al. (1996)
BG-1 hER(E)+Luc(S)	Benz[*a*]anthracene	56-55-3	pos.						Xenobiotic Detection Systems (2001)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Benz[*a*]anthracene	56-55-3	pos.		4				Clemons et al. (1998)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Benz[*a*]anthracene	56-55-3	weak						Fertuck et al. (2001b)
MCF-7 hER(E)+CP(F)	Benz[*a*]anthracene	56-55-3				neg.	pos.		Arcaro et al. (1999b)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	Benz[*a*]anthracene	56-55-3	weak						Fertuck et al. (2001b)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Benzo[*a*]carbazole	239-01-0	weak						Fertuck et al. (2001b)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	Benzo[*a*]carbazole	239-01-0	weak						Fertuck et al. (2001b)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Benzo[*c*]carbazole		weak						Fertuck et al. (2001b)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	Benzo[*c*]carbazole		weak						Fertuck et al. (2001b)
MCF-7 hER(E)+CP(F)	Benzo[*b*]fluoranthene	205-99-2				neg.	pos.		Arcaro et al. (1999b)
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	Benzo[*b*]fluoranthene	205-99-2	neg.				neg.	94	Tran et al. (1996)
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	Benzo[*b*]fluoranthene	205-99-2					neg.		Tran et al. (1996)
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	Benzo[*j*]fluoranthene	205-82-3	neg.				neg.	100	Tran et al. (1996)
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	Benzo[*j*]fluoranthene	205-82-3					neg.		Tran et al. (1996)
BG-1 hER(E)+Luc(S)	Benzo[*k*]fluoranthene	207-08-9	pos.						Xenobiotic Detection Systems (2001)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
BG-1 hER(E)+Luc(S)	Benzo[*k*]fluoranthene	207-08-9	pos.						Xenobiotic Detection Systems (2001)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Benzo[*k*]fluoranthene	207-08-9	neg.		0				Clemons et al. (1998)
MCF-7 hER(E)+CP(F)	Benzo[*k*]fluoranthene	207-08-9				neg.	pos.		Arcaro et al. (1999b)
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	Benzo[*k*]fluoranthene	207-08-9	neg.				neg.	101	Tran et al. (1996)
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	Benzo[*k*]fluoranthene	207-08-9					neg.		Tran et al. (1996)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Benzo[*b*]fluorene	243-17-4	weak						Fertuck et al. (2001b)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	Benzo[*b*]fluorene	243-17-4	weak						Fertuck et al. (2001b)
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	Benzo[*b*]fluorene	243-17-4	neg.				pos.	78	Tran et al. (1996)
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	Benzo[*b*]fluorene	243-17-4					neg.	97	Tran et al. (1996)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Benzo[*b*]naphtho[*2,1-d*]thiophene	239-35-0	weak						Fertuck et al. (2001b)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	Benzo[*b*]naphtho[*2,1-d*]thiophene	239-35-0	weak						Fertuck et al. (2001b)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Benzo[*b*]naphtho[*2,3-d*]thiophene	243-46-9	weak						Fertuck et al. (2001b)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	Benzo[*b*]naphtho[*2,3-d*]thiophene	243-46-9	weak						Fertuck et al. (2001b)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Benzo[*ghi*]perylene	191-24-2	neg.		0				Clemons et al. (1998)
MCF-7 hER(E)+CP(F)	Benzo[*ghi*]perylene	191-24-2				neg.	neg.		Arcaro et al. (1999b)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Benzo[*c*]phenanthrene	195-19-7	neg.						Fertuck et al. (2001b)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	Benzo[*c*]phenanthrene	195-19-7	neg.						Fertuck et al. (2001b)
ZR-75 hER(E)+CP	Benzophenone	119-61-9				neg.			Jobling et al. (1995)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Benzophenone-1	131-56-6	pos.	0.0003					Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Benzophenone-2	131-55-5	pos.	0.0001					Miller et al. (2001)
MCF-7(Bos) hER(E)+CP	Benzophenone-3	131-57-7		105	3.73	pos.			Schlumpf et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Benzophenone-3	131-57-7	pos.	<0.00001					Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Benzophenone-4	4065-45-6	neg.						Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Benzophenone-6	131-54-4	pos.	<0.00000001					Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Benzophenone-7	85-19-8	pos.	<0.000003					Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Benzophenone-8	131-53-3	neg.						Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Benzophenone-12	1843-05-6	neg.						Miller et al. (2001)
BG-1 hER(E)+Luc(S)	Benzo[*a*]pyrene	50-32-8	pos.						Xenobiotic Detection Systems (2001)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Benzo[*a*]pyrene	50-32-8	pos.	0.43					Charles et al. (2000b)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Benzo[*a*]pyrene	50-32-8	pos.		1.3				Clemons et al. (1998)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Benzo[*a*]pyrene	50-32-8	wi				neg.		Fertuck et al. (2001a)
MCF-7 hER(E)+CP(F)	Benzo[*a*]pyrene	50-32-8				neg.	pos.		Arcaro et al. (1999b)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	Benzo[*a*]pyrene	50-32-8		0.039	0.33		neg.		Fertuck et al. (2001a)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	Benzo[*a*]pyrene	50-32-8	neg.				pos.	77	Tran et al. (1996)
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	Benzo[*a*]pyrene	50-32-8					neg.	90	Tran et al. (1996)
MCF-7 hER(E)+CP(F)	Benzo[*e*]pyrene	192-97-2				neg.	pos.		Arcaro et al. (1999b)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Benzo[*a*]pyrene-1,6-dione	3067-13-8	ni						Fertuck et al. (2001a)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	Benzo[*a*]pyrene-1,6-dione	3067-13-8	ni						Fertuck et al. (2001a)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Benzo[*a*]pyrene-3,6-dione	3067-14-9	ni						Fertuck et al. (2001a)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	Benzo[*a*]pyrene-3,6-dione	3067-14-9	ni						Fertuck et al. (2001a)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Benzo[*a*]pyrene-6,12-dione	3067-12-7	ni						Fertuck et al. (2001a)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	Benzo[*a*]pyrene-6,12-dione	3067-12-7	ni						Fertuck et al. (2001a)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Benzylparaben	94-18-8	pos.	0.00025					Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Benzyl salicylate	118-58-1	pos.	<0.000002					Miller et al. (2001)
T47D hER(E)+CP	Betulin	473-98-3				pos.			Mellanen et al. (1996)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Bifenix	42576-02-3	pos.	16.97					Petit et al. (1997)
HEK293 hERα(T)+Luc(T)+β-gal(T)	Biochanin A	491-80-5	pos.	36			neg.		Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	Biochanin A	491-80-5	pos.	53			neg.		Kuiper et al. (1998)
HeLa hER(T)+CAT(T)	Biochanin A	491-80-5	pos.	50	2				Miksicek (1994)
HeLa mER(T)+CAT(T)*	Biochanin A	491-80-5	pos.	7.4x (2 µM)					Makela et al. (1994)
MCF-7 hER(E)+CP	Biochanin A	491-80-5				pos.	neg.		Makela et al. (1994)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
MCF-7(M) hER(E)+Luc(T)+β-gal(T)	Biochanin A	491-80-5	pos.	107			pos.		Collins-Burow et al. (2000)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Biochanin A	491-80-5	pos.	0.0091					Coldham et al. (1997)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Biochanin A	491-80-5	pos.	96.89					Petit et al. (1997)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Biochanin A	491-80-5	neg.				pos.		Morito et al. (2001b)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Biochanin A	491-80-5	neg.		0.2		pos.		Morito et al. (2001b)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	2,2'-Biphenol	1806-29-7	neg.						Miller et al. (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	2,2'-Biphenol	1806-29-7	pos.	18.17					Petit et al. (1997)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Biphenyl	92-52-4	pos.	52.53					Petit et al. (1997)
HeLa mER(T)+CAT(T)	3,4-Biphenyldiol		pos.	41.6					Garner et al. (1999)
HEK293 hERα(T)+Luc(T)+β-gal(T)	4,4'-Biphenyldiol	92-88-6	pos.	53					Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	4,4'-Biphenyldiol	92-88-6	pos.	72					Kuiper et al. (1998)
MCF-7 hER(E)+CP	4,4'-Biphenyldiol	92-88-6				pos.			Soto et al. (1995)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	4,4'-Biphenyldiol	92-88-6	pos.	0.0001					Miller et al. (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	4,4'-Biphenyldiol	92-88-6	pos.	96.06					Petit et al. (1997)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	2-Biphenylol	90-43-7	pos.	0.0000005					Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	2-Biphenylol	90-43-7	pos.	0.000000001	107				Vinggaard et al. (2000)
Yeast(*S.cer.*) hER(S)+β-gal(S)	2-Biphenylol	90-43-7	pos.	0.000002	100				Routledge and Sumpter (1997)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	2-Biphenylol	90-43-7	pos.	91.96					Petit et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	3-Biphenylol	580-51-8	pos.	0.000005	40				Routledge and Sumpter (1997)

Sorted by Substance and Assay

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	3-Biphenylol	580-51-8	pos.	96.92					Petit et al. (1997)
MCF-7 hER(E)+CP	4-Biphenylol	92-69-3				pos.			Korner et al. (1998)
MCF-7 hER(E)+CP	4-Biphenylol	92-69-3				pos.			Soto et al. (1995)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	4-Biphenylol	92-69-3	pos.	0.0001					Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	4-Biphenylol	92-69-3	pos.	0.00002	1				Vinggaard et al. (2000)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-Biphenylol	92-69-3	pos.	0.00002	10				Routledge and Sumpter (1997)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	4-Biphenylol	92-69-3	pos.	139.02					Petit et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Bis(2-*n*- butoxyethyl) phthalate	117-83-9	neg.						Harris et al. (1997)
MCF-7(E3) hER(E)+CP	Bisdesoxyestradiol	1217-09-0			201	pos.			Wiese et al. (1997)
Yeast(*S.cer* .) hERα(S)+β-gal(S)	Bisdesoxyestradiol	1217-09-0	pos.	0.00003	1.64				Elsby et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Bis(ethoxyethyl) phthalate	605-54-9	neg.						Harris et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Bis[2-(2-ethoxyethyoxy)ethyl] phthalate		neg.						Harris et al. (1997)
ZR-75 hER(E)+CP	Bis(2-ethylhexyl)adipate	103-23-1				neg.			Jobling et al. (1995)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Bis(2-ethylhexyl) hexahydrophthalate		neg.						Harris et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Bis(2-ethylhexyl)isophthalate	137-89-3	neg.						Harris et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Bis(2-ethylhexyl)isophthalate	137-89-3	neg.						Harris et al. (1997)
CHO-K1 hERα(T)+Luc(T)†	Bis(2-ethylhexyl)phthalate	117-81-7	neg.		0				Otsuka Pharmaceutical (2001)
HeLa hERαdef(S)+Luc(S)	Bis(2-ethylhexyl)phthalate	117-81-7	neg.	0					Zacharewski et al. (1998)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Bis(2-ethylhexyl)phthalate	117-81-7		0					Zacharewski et al. (1998)

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MCF-7 hER(E)+CP	Bis(2-ethylhexyl)phthalate	117-81-7				neg.			Harris et al. (1997)
MCF-7 hER(E)+CP	Bis(2-ethylhexyl)phthalate	117-81-7				neg.			Jones et al. (1998)
MCF-7 hER(E)+Luc(T)+β-gal(T)	Bis(2-ethylhexyl)phthalate	117-81-7	weak						Jobling et al. (1995)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Bis(2-ethylhexyl)phthalate	117-81-7	neg.	0					Coldham et al. (1997)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Bis(2-ethylhexyl)phthalate	117-81-7	pos.	17.67					Petit et al. (1997)
ZR-75 hER(E)+CP	Bis(2-ethylhexyl)phthalate	117-81-7				neg.			Harris et al. (1997)
ZR-75 hER(E)+CP	Bis(2-ethylhexyl)phthalate	117-81-7				neg.			Jobling et al. (1995)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Bis(2-ethylhexyl)phthalate	117-81-7	neg.						Harris et al. (1997)
HepG2 hERα(T)+Luc(T)+β-gal(T)	Bis(4-hydroxyphenyl)ethane	6052-84-2	pos.	85	1.6		neg.	110	Gaido et al. (2000)
HepG2 hERβ(T)+Luc(T)+β-gal(T)	Bis(4-hydroxyphenyl)ethane	6052-84-2	pos.	70	2.1		neg.	85µM	Gaido et al. (2000)
HepG2 hERα(T)+Luc(T)+β-gal(T)	Bis(4-hydroxyphenyl)methane	620-92-8	pos.	55	1.5		neg.	120	Gaido et al. (2000)
HepG2 hERβ(T)+Luc(T)+β-gal(T)	Bis(4-hydroxyphenyl)methane	620-92-8	pos.	55	2		neg.	120µM	Gaido et al. (2000)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Bis(4-hydroxyphenyl)methane	620-92-8	pos.	0.0001					Miller et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Bis(methoxyethyl)phthalate	117-82-8	neg.						Harris et al. (1997)
BG-1 hER(E)+Luc(S)	Bisphenol A	80-05-7	pos.	0.0044	0.23				Xenobiotic Detection Systems (2001)
BG-1 hER(E)+Luc(T)	Bisphenol A	80-05-7	pos.	36					Rogers and Denison (2000)
CHO-K1 hERα(S)+Luc(S)†	Bisphenol A	80-05-7	pos.		0.348				Otsuka Pharmaceutical (2001)
CHO-K1 hERα(T)+Luc(T)†	Bisphenol A	80-05-7	pos.		0.253				Otsuka Pharmaceutical (2001)
HEK293 hERα(T)+Luc(T)+β-gal(T)	Bisphenol A	80-05-7	pos.	50					Kuiper et al. (1998)

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HEK293 hERβ(T)+Luc(T)+β-gal(T)	Bisphenol A	80-05-7	pos.	41					Kuiper et al. (1998)
HeLa hERα(T)+Luc(T)	Bisphenol A	80-05-7	pos.						Tarumi et al. (2000)
HepG2 hERα(T)+Luc(T)+β-gal(T)	Bisphenol A	80-05-7	pos.	100	0.64		neg.	115	Gaido et al. (2000)
HepG2 hERα(T)+Luc(T)+β-gal(T)	Bisphenol A	80-05-7	pos.	4.5	0.218				Gould et al. (1998)
HepG2 hERβ(T)+Luc(T)+β-gal(T)	Bisphenol A	80-05-7	pos.	45	0.89		neg.	90µM	Gaido et al. (2000)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Bisphenol A	80-05-7		0.0001	0.71				Matthews et al. (2001)
MCF-7 hERβdef(T)+Luc(T)+β-gal(T)	Bisphenol A	80-05-7		0.0002	0.45				Matthews et al. (2001)
MCF-7 hER(E)+CP	Bisphenol A	80-05-7				pos.			Dodge et al. (1996)
MCF-7 hER(E)+CP	Bisphenol A	80-05-7				pos.			Korner et al. (1998)
MCF-7 hER(E)+CP	Bisphenol A	80-05-7				pos.			Morito et al. (2001a)
MCF-7 hER(E)+CP	Bisphenol A	80-05-7				pos.			Nakagawa and Suzuki (2001)
MCF-7 hER(E)+CP	Bisphenol A	80-05-7				pos.			Soto et al. (1995)
MCF-7 hER(E)+Luc(T)	Bisphenol A	80-05-7	pos.						Yoshihara et al. (2001)
MCF-7(BUS) hER(E)+CP	Bisphenol A	80-05-7				pos.			Schafer et al. (1999)
T47D hER(E)+CP	Bisphenol A	80-05-7				pos.			Schafer et al. (1999)
T47D hER(E)+Luc(S)	Bisphenol A	80-05-7	pos.	0.0078	0.77				Legler et al. (1999)
T47D hER(E)+Luc(S)	Bisphenol A	80-05-7	pos.		0.000033				Meerts et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Bisphenol A	80-05-7	pos.	0.0001					Beresford et al. (2000)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Bisphenol A	80-05-7	pos.		0.00072-0.854				De Boever et al. (2001)
Yeast(*S.cer* .) hERα(S)+β-gal(S)	Bisphenol A	80-05-7	pos.	0.00007	0.71				Elsby et al. (2001)

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Yeast(*S.cer.*) hERα(S)+β-gal(S)	Bisphenol A	80-05-7	pos.	0.0001					Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Bisphenol A	80-05-7	pos.						Yoshihara et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)+S9*	Bisphenol A	80-05-7	pos.						Yoshihara et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Bisphenol A	80-05-7	pos.	0.000026					Harris et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Bisphenol A	80-05-7	pos.	0.00003	3.9				Rajapakse et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Bisphenol A	80-05-7	pos.	0.0003	0.114				Routledge and Sumpter (1996)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Bisphenol A	80-05-7	pos.	0.005					Coldham et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Bisphenol A	80-05-7	pos.	0.00007	3.4				Gaido et al. (1997)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Bisphenol A	80-05-7	pos.		0.1				Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Bisphenol A	80-05-7	pos.		0.1				Morito et al. (2001a)
ZR-75-1 hER(E)+CP	Bisphenol A	80-05-7				pos.			Schafer et al. (1999)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Bisphenol A	80-05-7	pos.	0.000025	0.8				Vinggaard et al. (2000)
HeLa hERα(T)+Luc(T)	Bisphenol A dimethacrylate	3253-39-2	pos.						Tarumi et al. (2000)
MCF-7 hER(E)+CP	Bisphenol A dimethacrylate	3253-39-2				pos.			Korner et al. (1998)
MCF-7(BUS) hER(E)+CP	Bisphenol A dimethacrylate	3253-39-2				pos.			Schafer et al. (1999)
T47D hER(E)+CP	Bisphenol A dimethacrylate	3253-39-2				pos.			Schafer et al. (1999)
ZR-75-1 hER(E)+CP	Bisphenol A dimethacrylate	3253-39-2				neg.			Schafer et al. (1999)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Bisphenol A glucuronide			0	0				Matthews et al. (2001)
MCF-7 hERβdef(T)+Luc(T)+β-gal(T)	Bisphenol A glucuronide			0	0				Matthews et al. (2001)

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CHO-K1 hERα(S)+Luc(S)†	Bisphenol B	77-40-7	pos.		0.114				Otsuka Pharmaceutical (2001)
CHO-K1 hERα(T)+Luc(T)†	Bisphenol B	77-40-7	pos.		0.0624				Otsuka Pharmaceutical (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Bisphenol B	77-40-7	pos.						Yoshihara et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)+S9*	Bisphenol B	77-40-7	pos.						Yoshihara et al. (2001)
CHO-K1 hERα(S)+Luc(S)†	2,2-Bis(*p* -hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	pos.		0.0299				Otsuka Pharmaceutical (2001)
CHO-K1 hERα(T)+Luc(T)†	2,2-Bis(*p* -hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	pos.		0.0696				Otsuka Pharmaceutical (2001)
ELT-3 hER(T)+Luc(T)+β-gal(T)	2,2-Bis(*p* -hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	pos.	23.38					Hodges et al. (2000)
HeLa hERα(T)+Luc(T)+β-gal(T)	2,2-Bis(*p* -hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	pos.	0.03	0.03		pos.		Gaido et al. (1999)
HeLa hERβ(T)+Luc(T)+β-gal(T)	2,2-Bis(*p* -hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	pos.	0.005	0.1		pos.		Gaido et al. (1999)
HeLa mER(T)+CAT(T)*	2,2-Bis(*p* -hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	pos.	67 (1 µM)					Shelby et al. (1996)
HepG2 hERα(T)+Luc(T)+β-gal(T)	2,2-Bis(*p* -hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	pos.	7.84	0.051		neg.		Gaido et al. (1999)
HepG2 hERα(T)+Luc(T)+β-gal(T)	2,2-Bis(*p* -hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	pos.	100	0.051		neg.	110	Gaido et al. (2000)
HepG2 hERβ(T)+Luc(T)+β-gal(T)	2,2-Bis(*p* -hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	pos.	50	0.03		pos.		Gaido et al. (1999)
HepG2 hERβ(T)+Luc(T)+β-gal(T)	2,2-Bis(*p* -hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	pos.	80	0.01		pos.		Gaido et al. (1999)
HepG2 hERβ(T)+Luc(T)+β-gal(T)	2,2-Bis(*p* -hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	pos.	30			pos.	35µM	Gaido et al. (2000)
HepG2 rERα(T)+Luc(T)+β-gal(T)	2,2-Bis(*p* -hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	pos.	10	0.01		neg.		Gaido et al. (1999)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	2,2-Bis(*p* -hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	pos.	0.19	0.05				Charles et al. (2000a)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	2,2-Bis(*p* -hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	pos.	0.0001					Beresford et al. (2000)

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Yeast(*S.cer* .) hERα(S)+β-gal(S)	2,2-Bis(*p* -hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	pos.	0.0004	0.128				Elsby et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	2,2-Bis(*p* -hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	pos.	0.003	0.05				Odum et al. (1997)
MCF-7 hER(E)+CP	6-Bromo-2-naphthol	15231-91-1				neg.			Soto et al. (1995)
MCF-7(E3) hER(E)+CP	Bromopropylate	18181-80-1				neg.			Vinggaard et al. (1999)
T47D hER(E)+Luc(S)	2-Bromo-4-(2,4,6-tribromophenoxy)phenol		neg.		0.00002		neg.		Meerts et al. (2001)
MCF-7 hER(E)+CP	4-Butoxyphenol	122-94-1				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP	Butylate	2008-41-5				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP	Butylated hydroxyanisole	25013-16-5				neg.			Jones et al. (1998)
MCF-7 hER(E)+CP	Butylated hydroxyanisole	25013-16-5				pos.			Korner et al. (1998)
MCF-7 hER(E)+CP	Butylated hydroxyanisole	25013-16-5				pos.			Soto et al. (1995)
MCF-7 hER(E)+Luc(T)+β-gal(T)	Butylated hydroxyanisole	25013-16-5	pos.						Jobling et al. (1995)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Butylated hydroxyanisole	25013-16-5	pos.	<0.0000005					Miller et al. (2001)
ZR-75 hER(E)+CP	Butylated hydroxyanisole	25013-16-5				pos.			Jobling et al. (1995)
MCF-7 hER(E)+CP	Butylated hydroxytoluene	128-37-0				neg.			Jones et al. (1998)
MCF-7 hER(E)+CP	Butylated hydroxytoluene	128-37-0				neg.			Soto et al. (1995)
MCF-7 hER(E)+Luc(T)+β-gal(T)	Butylated hydroxytoluene	128-37-0	weak						Jobling et al. (1995)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Butylated hydroxytoluene	128-37-0	pos.	<0.0000001					Miller et al. (2001)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Butylated hydroxytoluene	128-37-0	neg.	0					Coldham et al. (1997)
ZR-75 hER(E)+CP	Butylated hydroxytoluene	128-37-0				pos.			Jobling et al. (1995)
ZR-75 hER(E)+CP	*n* -Butylbenzene	104-51-8				neg.			Jobling et al. (1995)
CHO-K1 hERα(T)+Luc(T)†	Butyl benzyl phthalate	85-68-7	neg.		0				Otsuka Pharmaceutical (2001)
HeLa hERαdef(S)+Luc(S)	Butyl benzyl phthalate	85-68-7	pos.	34					Zacharewski et al. (1998)

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MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Butyl benzyl phthalate	85-68-7		46					Zacharewski et al. (1998)
MCF-7 hER(E)+CP	Butyl benzyl phthalate	85-68-7				pos.			Harris et al. (1997)
MCF-7 hER(E)+CP	Butyl benzyl phthalate	85-68-7				neg.			Jones et al. (1998)
MCF-7 hER(E)+CP	Butyl benzyl phthalate	85-68-7				pos.			Korner et al. (1998)
MCF-7 hER(E)+CP	Butyl benzyl phthalate	85-68-7				pos.			Soto et al. (1995)
MCF-7 hER(E)+Luc(T)+β-gal(T)	Butyl benzyl phthalate	85-68-7	pos.				neg.		Jobling et al. (1995)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Butyl benzyl phthalate	85-68-7	pos.	0.0000025					Beresford et al. (2000)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Butyl benzyl phthalate	85-68-7	pos.	0.0004					Coldham et al. (1997)
ZR-75 hER(E)+CP	Butyl benzyl phthalate	85-68-7				pos.			Harris et al. (1997)
ZR-75 hER(E)+CP	Butyl benzyl phthalate	85-68-7				pos.			Jobling et al. (1995)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Butyl benzyl phthalate	85-68-7	pos.	0.00001					Harris et al. (1997)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	4-*tert* -Butylcatechol	98-29-3	pos.	0.000003					Miller et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Butyl cyclohexyl phthalate	84-64-0	neg.						Harris et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Butyl decyl phthalate	89-19-0	neg.						Harris et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Butyl 2-ethylhexyl phthalate	85-69-8	neg.						Harris et al. (1997)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	5-*tert* -Butyl-4-hydroxy-2-methyl-phenyl sulfide	96-69-5	neg.						Miller et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Butyl isodecyl phthalate	42343-36-2	neg.						Harris et al. (1997)
MCF-7(Bos) hER(E)+CP	Butyl methoxydibenzoylmethane	70356-09-1		21.01		neg.			Schlumpf et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Butyl octyl phthalate	84-78-6	neg.						Harris et al. (1997)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Butylparaben	94-26-8	pos.	0.0001					Miller et al. (2001)

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Yeast(*S.cer.*) hER(S)+β-gal(S)	2-*tert* -Butylphenol	88-18-6	neg.	0					Routledge and Sumpter (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	3-*tert* -Butylphenol	585-34-2	neg.						Routledge and Sumpter (1997)
MCF-7 hER(E)+CP	4-*sec* -Butylphenol	99-71-8				pos.			Soto et al. (1995)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-*sec* -Butylphenol	99-71-8	pos.	0.0000003	666				Routledge and Sumpter (1997)
MCF-7 hER(E)+CP	4-*tert* -Butylphenol	98-54-4				pos.			Korner et al. (1998)
MCF-7 hER(E)+CP	4-*tert* -Butylphenol	98-54-4				pos.			Soto et al. (1995)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	4-*tert* -Butylphenol	98-54-4	variable						Beresford et al. (2000)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	4-*tert* -Butylphenol	98-54-4	pos.	0.0000003					Miller et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-*tert* -Butylphenol	98-54-4	pos.	0.0000008	250				Routledge and Sumpter (1997)
MCF-7 hER(E)+CP	Caffeine	58-08-2				neg.			Jones et al. (1998)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Captan	133-06-2	pos.	13.93					Petit et al. (1997)
Ishikawa hER(T)+Luc(T)+β-gal(T)	Carbaryl	63-25-2	neg.	0.07			pos.	34%	Klotz et al. (1997)
MCF-7 hER(E)+CP	Carbaryl	63-25-2				neg.			Soto et al. (1995)
MCF-7 hER(E)+Luc(T)+β-gal(T)	Carbaryl	63-25-2		0.07				29%	Klotz et al. (1997)
BG-1 hER(E)+Luc(S)	Carbazole	86-74-8	neg.						Xenobiotic Detection Systems (2001)
MCF-7 hER(E)+CP	Carbofuran	1563-66-2				neg.			Soto et al. (1995)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Carbofuran	1563-66-2	pos.	16.82					Petit et al. (1997)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Carbosulfan	55285-14-8	pos.	14.09					Petit et al. (1997)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Carvacrol	499-75-2	neg.						Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Catechol	120-80-9	neg.						Miller et al. (2001)

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MCF-7 hER(E)+CP	Chlordane	57-74-9				neg.			Soto et al. (1995)
T47D hER(E)+Luc(S)	Chlordane	57-74-9	pos.	0.00096	6.24				Legler et al. (1999)
Yeast(*S.cer.* BJ2168) mER(S)+β-gal(S)	Chlordane	57-74-9	pos.	6x (25 µM)*					Ramamoorthy et al. (1997a)
Yeast(*S.cer.* BJ2168) mER(S)+β-gal(S)	Chlordane	57-74-9	pos.	0.6x (100 µM)*					Ramamoorthy et al. (1997a)
BG-1 hER(E)+Luc(S)	α-Chlordane	5103-71-9	pos.						Xenobiotic Detection Systems (2001)
BG-1 hER(E)+Luc(S)	γ-Chlordane	5103-74-2	pos.						Xenobiotic Detection Systems (2001)
MCF-7 hER(E)+CP	Chlordimeform	6164-98-3				neg.			Soto et al. (1995)
MCF-7(E3) hER(E)+CP	Chlorfenvinphos	470-90-6				neg.			Vinggaard et al. (1999)
MCF-7(E3) hER(E)+CP	Chlorobenzilate	510-15-6				neg.			Vinggaard et al. (1999)
MCF-7 hER(E)+CP	2-Chlorobiphenyl	2051-60-7				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP(F)	2-Chlorobiphenyl	2051-60-7				pos.	neg.		Gierthy et al. (1997)
MCF-7 hER(E)+CP	4-Chlorobiphenyl	2051-62-9				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP(F)	4-Chlorobiphenyl	2051-62-9				pos.	pos.		Gierthy et al. (1997)
MCF-7 hER(E)+CP(F)	2-Chloro-4-biphenylol	23719-22-4				pos.	neg.		Gierthy et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	2'-Chloro-4-biphenylol		pos.	0.0037					Coldham et al. (1997)
MCF-7 hER(E)+CP(F)	4-Chloro-4'-biphenylol	28034-99-3				pos.	neg.		Gierthy et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	4-Chloro-4'-biphenylol	28034-99-3	pos.	0.06					Coldham et al. (1997)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	4-Chloro-3,5-dimethylphenol	88-04-0	pos.	0.000001					Miller et al. (2001)
MCF-7 hER(E)+CP	11β-Chloromethylestradiol	71794-60-0				pos.			Soto et al. (1995)
MCF-7 hER(E)+CP	4-Chloro-2-methylphenol	1570-64-5				pos.			Korner et al. (1998)
MCF-7 hER(E)+CP	4-Chloro-3-methylphenol	59-50-7				pos.			Korner et al. (1998)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	4-Chloro-3-methylphenol	59-50-7	pos.	0.0000003					Miller et al. (2001)

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Yeast(*S.cer.*) hERα(S)+β-gal(S)	4-Chloro-3-methylphenol	59-50-7	pos.	0.0000000005	378				Vinggaard et al. (2000)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Chlorosulfuron	64902-72-3	pos.	14.98					Petit et al. (1997)
MCF-7 hER(E)+CP	Chlorothalonil	1897-45-6				neg.			Soto et al. (1995)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Chlorothalonil	1897-45-6	pos.	11.33					Petit et al. (1997)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Chlorothymol	89-68-9	pos.	0.0000025					Miller et al. (2001)
MCF-7 hER(E)+CP	Chlorpyrifos	2921-88-2				neg.			Soto et al. (1995)
MCF-7(E3) hER(E)+CP	Chlorpyrifos	2921-88-2				neg.			Vinggaard et al. (1999)
MCF-7 hER(E)+CP	Cholesterol	57-88-5				neg.			Jones et al. (1998)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Cholesterol	57-88-5	neg.	0					Coldham et al. (1997)
BG-1 hER(E)+Luc(S)	Chrysene	218-01-9	pos.						Xenobiotic Detection Systems (2001)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Chrysene	218-01-9	pos.		5.5				Clemons et al. (1998)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Chrysene	218-01-9	neg.						Fertuck et al. (2001b)
MCF-7 hER(E)+CP(F)	Chrysene	218-01-9				neg.	pos.		Arcaro et al. (1999b)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	Chrysene	218-01-9	neg.						Fertuck et al. (2001b)
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	Chrysene	218-01-9	neg.				neg.	97	Tran et al. (1996)
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	Chrysene	218-01-9					neg.		Tran et al. (1996)
HEK293 hERα(T)+Luc(T)+β-gal(T)	Chrysin	480-40-0	neg.	1			neg.		Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	Chrysin	480-40-0	pos.	2			neg.		Kuiper et al. (1998)
MCF-7(M) hER(E)+Luc(T)+β-gal(T)	Chrysin	480-40-0	pos.	125			pos.		Collins-Burow et al. (2000)

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MCF-7(M) hER(E)+CP	Chrysin	480-40-0					pos.		Collins-Burow et al. (2000)
T47D hER(E)+CP	Citrostadienol					neg.			Mellanen et al. (1996)
CHO-K1 hERα(T)+Luc(T)†	*trans* -Clomiphene	911-45-5	neg.		0				Otsuka Pharmaceutical (2001)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	*trans* -Clomiphene	911-45-5	pos.	0.00002	9.97				Gaido et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Cocobetaine	68411-97-2	neg.						Routledge and Sumpter (1996)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Coconut amido betaine		neg.						Routledge and Sumpter (1996)
MCF-7 hER(E)+CP	Corn oil	8001-30-7				neg.			Go et al. (1999)
CHO-K1 hERα(T)+Luc(T)†	Corticosterone	50-22-6	neg.		0				Otsuka Pharmaceutical (2001)
CHO-K1 hERα(T)+Luc(T)†	Cortisol	50-23-7	neg.		0				Otsuka Pharmaceutical (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Cortisol	50-23-7	neg.						Routledge and Sumpter (1996)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Cortisol	50-23-7	neg.	0					Routledge and Sumpter (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Cortisol	50-23-7	neg.	0					Coldham et al. (1997)
BG-1 hER(E)+Luc(S)	Coumestrol	479-13-0	pos.	0.1	0.01				Xenobiotic Detection Systems (2001)
CHO-K1 hERα(T)+Luc(T)†	Coumestrol	479-13-0	pos.		0.0168				Otsuka Pharmaceutical (2001)
HEK293 hERα(T)+Luc(T)+β-gal(T)	Coumestrol	479-13-0	pos.	102			neg.		Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	Coumestrol	479-13-0	pos.	98			neg.		Kuiper et al. (1998)
HeLa hER(T)+CAT(T)	Coumestrol	479-13-0	pos.	153.75	0.015				Miksicek (1994)
HeLa mER(T)+CAT(T)	Coumestrol	479-13-0		111		pos.			Makela et al. (1994)
HeLa mER(T)+CAT(T)*	Coumestrol	479-13-0	pos.	60x (100 nM)					Makela et al. (1994)
MCF-7 hER(E)+CP	Coumestrol	479-13-0				pos.			Dodge et al. (1996)

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MCF-7 hER(E)+CP	Coumestrol	479-13-0				pos.	neg.		Makela et al. (1994)
MCF-7 hER(E)+CP	Coumestrol	479-13-0				pos.			Soto et al. (1995)
MCF-7(M) hER(E)+Luc(T)+β-gal(T)	Coumestrol	479-13-0	pos.	56			pos.		Collins-Burow et al. (2000)
T47D hER(E)+CP	Coumestrol	479-13-0				pos.	neg.		Makela et al. (1994)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Coumestrol	479-13-0	pos.	0.67					Coldham et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Coumestrol	479-13-0	pos.	0.013	0.0174				Gaido et al. (1997)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Coumestrol	479-13-0	pos.		0.04		neg.		Morito et al. (2001b)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Coumestrol	479-13-0	pos.		0.02		neg.		Morito et al. (2001b)
BG-1 hER(E)+Luc(S)	Creosote	8001-58-9	neg.						Xenobiotic Detection Systems (2001)
BG-1 hER(E)+Luc(S)	*o* -Cresol	95-48-7	neg.						Xenobiotic Detection Systems (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	*o* -Cresol	95-48-7	neg.						Miller et al. (2001)
BG-1 hER(E)+Luc(S)	*p* -Cresol	106-44-5	neg.						Xenobiotic Detection Systems (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	*p* -Cresol	106-44-5	neg.						Miller et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	*p* -Cresol	106-44-5	neg.						Routledge and Sumpter (1997)
BG-1 hER(E)+Luc(S)	Cumene	98-82-8	neg.						Xenobiotic Detection Systems (2001)
CHO-K1 hERα(S)+Luc(S)†	*p* -Cumylphenol	599-64-4	pos.		0.395				Otsuka Pharmaceutical (2001)
CHO-K1 hERα(T)+Luc(T)†	*p* -Cumylphenol	599-64-4	pos.		0.248				Otsuka Pharmaceutical (2001)
HeLa hER(T)+CAT(T)	Curcumin	458-37-7	neg.	5					Miksicek (1994)
MCF-7 hER(E)+CP	Cyanazine	21725-46-2				neg.			Soto et al. (1995)

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CHO-K1 hERα(T)+Luc(T)†	Cycloprothrin	63935-38-6	pos.						Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Cycloprothrin	63935-38-6				neg.			Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Cycloprothrin	63935-38-6				neg.			Otsuka Pharmaceutical (2001)
CHO-K1 hERα(T)+Luc(T)†	Cyfluthrin	68359-37-5	pos.						Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Cyfluthrin	68359-37-5				pos.			Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Cyfluthrin	68359-37-5				pos.			Otsuka Pharmaceutical (2001)
CHO-K1 hERα(T)+Luc(T)†	Cyhalothrin	68085-85-8	pos.						Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Cyhalothrin	68085-85-8				pos.			Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Cyhalothrin	68085-85-8				pos.			Otsuka Pharmaceutical (2001)
BG-1 hER(E)+Luc(S)	*p* -Cymene	99-87-6	neg.						Xenobiotic Detection Systems (2001)
CHO-K1 hERα(T)+Luc(T)†	Cypermethrin	52315-07-8	neg.						Otsuka Pharmaceutical (2001)
HeLa hERα(T)+Luc(T)	Cypermethrin	52315-07-8	neg.	9.6					Sumida et al. (2001)
MCF-7 hER(E)+CP	Cypermethrin	52315-07-8				pos.			Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Cypermethrin	52315-07-8				pos.			Otsuka Pharmaceutical (2001)
CHO-K1 hERα(T)+Luc(T)†	Cyproterone acetate	427-51-0	neg.		0				Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Dacthal	1861-32-1				neg.			Soto et al. (1995)
BG-1 hER(E)+Luc(S)	Daidzein	486-66-8	pos.	0.002	0.49				Xenobiotic Detection Systems (2001)
BG-1 hER(E)+Luc(T)	Daidzein	486-66-8	pos.	44					Rogers and Denison (2000)
HEK293 hERα(T)+Luc(T)+β-gal(T)	Daidzein	486-66-8	pos.	97			neg.		Kuiper et al. (1998)

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HEK293 hERβ(T)+Luc(T)+β-gal(T)	Daidzein	486-66-8	pos.	80			neg.		Kuiper et al. (1998)
HeLa hER(T)+CAT(T)	Daidzein	486-66-8	pos.	95	0.09				Miksicek (1994)
MCF-7 hER(E)+CP	Daidzein	486-66-8			0.4	pos.			Ichikawa et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Daidzein	486-66-8	pos.	0.0013					Coldham et al. (1997)
MCF-7 hER(E)+CP	Daidzein	486-66-8				pos.			Morito et al. (2001a)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Daidzein	486-66-8	neg.						De Boever et al. (2001)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Daidzein	486-66-8	pos.		30				Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Daidzein	486-66-8	pos.		5				Morito et al. (2001a)
MCF-7 hER(E)+CP	Daidzin	552-66-9				pos.			Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Daidzin	552-66-9	pos.		100				Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Daidzin	552-66-9	neg.						Morito et al. (2001a)
Yeast(*S.cer* .939) hER(S)+β-gal(S)	*p,p* '-DDA	83-05-6	neg.	0					Chen et al. (1997)
Yeast(*S.cer.* CYT10-5d) hER(S)+β-gal(S)	*p,p* '-DDA	83-05-6	neg.	0					Chen et al. (1997)
MCF-7 hER(E)+Luc(T)+β-gal(T)	*o,p* '-DDD	53-19-0		0.43					Klotz et al. (1996)
Yeast(*S.cer* .939) hER(S)+β-gal(S)	*o,p* '-DDD	53-19-0	pos.	0.581					Chen et al. (1997)
Yeast(*S.cer.* BJ2407) hER(S)+β-gal(S)	*o,p* '-DDD	53-19-0		35x (10 µM)*			neg.		Klotz et al. (1996)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	*o,p* '-DDD	53-19-0	pos.	0.00000004	3320				Gaido et al. (1997)
Yeast(*S.cer.* CYT10-5d) hER(S)+β-gal(S)	*o,p* '-DDD	53-19-0	pos.	105x (10 µM)*					Chen et al. (1997)
BG-1 hER(E)+Luc(S)	*o,p* '-DDD	53-19-0	pos.						Xenobiotic Detection Systems (2001)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
CHO-K1 hERα(T)+Luc(T)†	*p,p* '-DDD	72-54-8	neg.		0				Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+Luc(T)+β-gal(T)	*p,p* '-DDD	72-54-8		0.87					Klotz et al. (1996)
Yeast(*S.cer.* BJ2407) hER(S)+β-gal(S)	*p,p* '-DDD	72-54-8		85x (10 µM)*			pos.	11uM	Klotz et al. (1996)
CHO-K1 hERα(T)+Luc(T)†	*o,p* '-DDE	3424-82-6	neg.		0				Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	*o,p* '-DDE	3424-82-6				pos.			Soto et al. (1995)
Yeast(*S.cer* .939) hER(S)+β-gal(S)	*o,p* '-DDE	3424-82-6	pos.	0.675					Chen et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	*o,p* '-DDE	3424-82-6	pos.	0.00004					Coldham et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	*o,p* '-DDE	3424-82-6	pos.	0.00000004	5340				Gaido et al. (1997)
Yeast(*S.cer.* CYT10-5d) hER(S)+β-gal(S)	*o,p* '-DDE	3424-82-6	pos.	101x (10 µM)*					Chen et al. (1997)
BG-1 hER(E)+Luc(S)	*p,p* '-DDE	72-55-9	neg.						Xenobiotic Detection Systems (2001)
CHO-K1 hERα(T)+Luc(T)†	*p,p* '-DDE	72-55-9	neg.		0				Otsuka Pharmaceutical (2001)
HepG2 hERα(T)+Luc(T)+β-gal(T)	*p,p* '-DDE	72-55-9	pos.	20			neg.	80	Gaido et al. (2000)
HepG2 hERβ(T)+Luc(T)+β-gal(T)	*p,p* '-DDE	72-55-9	pos.	10			neg.	100µM	Gaido et al. (2000)
MCF-7 hER(E)+CP	*p,p* '-DDE	72-55-9			3	pos.			Payne et al. (2001)
MCF-7 hER(E)+CP	*p,p* '-DDE	72-55-9				neg.			Soto et al. (1994)
MCF-7 hER(E)+CP	*p,p* '-DDE	72-55-9				pos.			Soto et al. (1995)
Yeast(*S.cer* .939) hER(S)+β-gal(S)	*p,p* '-DDE	72-55-9	neg.	0					Chen et al. (1997)
Yeast(*S.cer.* CYT10-5d) hER(S)+β-gal(S)	*p,p* '-DDE	72-55-9	neg.	0					Chen et al. (1997)
BG-1 hER(E)+Luc(T)	*o,p* '-DDT	789-02-6	pos.	56					Rogers and Denison (2000)

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CHO-K1 hERα(T)+Luc(T)†	*o,p* '-DDT	789-02-6	neg.		0				Otsuka Pharmaceutical (2001)
HEK293 hERα(T)+Luc(T)+β-gal(T)	*o,p* '-DDT	789-02-6	pos.	54					Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	*o,p* '-DDT	789-02-6	pos.	10					Kuiper et al. (1998)
HeLa mER(T)+CAT(T)*	*o,p* '-DDT	789-02-6	pos.	31 (10 µM)					Shelby et al. (1996)
MCF-7 hER(E)+CP	*o,p* '-DDT	789-02-6			0.8	pos.			Payne et al. (2001)
MCF-7 hER(E)+CP	*o,p* '-DDT	789-02-6				pos.			Soto et al. (1994)
MCF-7 hER(E)+CP	*o,p* '-DDT	789-02-6				pos.			Soto et al. (1995)
MCF-7 hER(E)+Luc(T)+β-gal(T)	*o,p* '-DDT	789-02-6		1.04					Klotz et al. (1996)
T47D hER(E)+Luc(S)	*o,p* '-DDT	789-02-6	pos.	0.0091	0.66				Legler et al. (1999)
Yeast(*S.cer.*) hER(S)+β-gal(S)	*o,p* '-DDT	789-02-6	pos.	0.00002					Harris et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	*o,p* '-DDT	789-02-6	pos.	0.00006	2.2				Rajapakse et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	*o,p* '-DDT	789-02-6	pos.	0.0002	0.18				Routledge and Sumpter (1996)
Yeast(*S.cer* .939) hER(S)+β-gal(S)	*o,p* '-DDT	789-02-6	pos.	0.655					Chen et al. (1997)
Yeast(*S.cer.* BJ2407) hER(S)+β-gal(S)	*o,p* '-DDT	789-02-6	pos.	0.001	0.2				Arnold et al. (1996)
Yeast(*S.cer.* BJ2407) hER(S)+β-gal(S)	*o,p* '-DDT	789-02-6		95x (10 uM)*			pos.	1uM	Klotz et al. (1996)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	*o,p* '-DDT	789-02-6	pos.	0.00011					Coldham et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	*o,p* '-DDT	789-02-6	pos.	0.0000001	1810				Gaido et al. (1997)
Yeast(*S.cer.* CYT10-5d) hER(S)+β-gal(S)	*o,p* '-DDT	789-02-6	pos.	110x (10 µM)*					Chen et al. (1997)
Yeast(*S.cer.* YRG-2) hERα(S)+β-gal(S)	*o,p* '-DDT	789-02-6	pos.	3.2x (10 µM)*					Lascombe et al. (2000)

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MCF-7(MELN41) hER(E)+Luc(S)	*o,p* '-DDT	789-02-6	pos.						Lascombe et al. (2000)
MCF-7(MELN41) hER(E)+Luc(S)	(-) *o,p* '-DDT	58633-26-4	pos.						Lascombe et al. (2000)
MCF-7(MELN41) hER(E)+Luc(S)	(+) *o,p* '-DDT	58633-27-5	pos.						Lascombe et al. (2000)
BG-1 hER(E)+Luc(S)	*p,p* '-DDT	50-29-3	pos.						Xenobiotic Detection Systems (2001)
HEK293 hERα(T)+Luc(T)+β-gal(T)	*p,p* '-DDT	50-29-3	pos.	7					Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	*p,p* '-DDT	50-29-3	pos.	2					Kuiper et al. (1998)
MCF-7 hER(E)+CP	*p,p* '-DDT	50-29-3			5	pos.			Payne et al. (2001)
MCF-7 hER(E)+CP	*p,p* '-DDT	50-29-3				pos.			Soto et al. (1995)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	*p,p* '-DDT	50-29-3	pos.		1.01-3.27				De Boever et al. (2001)
Yeast(*S.cer* .939) hER(S)+β-gal(S)	*p,p* '-DDT	50-29-3	pos.	0.495					Chen et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	*p,p* '-DDT	50-29-3	pos.	0.00003					Coldham et al. (1997)
Yeast(*S.cer.* CYT10-5d) hER(S)+β-gal(S)	*p,p* '-DDT	50-29-3	pos.	98x (10 µM)*					Chen et al. (1997)
MCF-7 hER(E)+CP	DDT (technical)	8017-34-3				pos.			Soto et al. (1994)
MCF-7 hER(E)+CP	DDT (technical)	8017-34-3				pos.			Soto et al. (1995)
MCF-7 hER(E)+CP	Decachlorobiphenyl	2051-24-3				neg.			Soto et al. (1995)
Yeast(*S.cer.*) hER(S)+β-gal(S)	2-*sec* -Decylphenol		neg.	0					Routledge and Sumpter (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-*sec* -Decylphenol		pos.	0.00001	2				Routledge and Sumpter (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Dehydroepiandrosterone	53-43-0	pos.	0.0018					Coldham et al. (1997)
CHO-K1 hERα(T)+Luc(T)†	Deltamethrin	52918-63-5	pos.						Otsuka Pharmaceutical (2001)

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MCF-7 hER(E)+CP	Deltamethrin	52918-63-5				pos.			Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Deltamethrin	52918-63-5				pos.			Otsuka Pharmaceutical (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Deltamethrin	52918-63-5	pos.	16.07					Petit et al. (1997)
Yeast(*S.cer* .188R1) hER(S)+β-gal(S)	Desethylatrazine		neg.				neg.		Graumann et al. (1999)
Yeast(*S.cer* .188R1) hER(S)+β-gal(S)	Desisopropylatrazine		neg.				neg.		Graumann et al. (1999)
BG-1 hER(E)+Luc(T)	Dexamethasone	50-02-2	pos.	9					Rogers and Denison (2000)
Yeast(*S.cer* .939) hER(S)+β-gal(S)	Dexamethasone	50-02-2	neg.	0					Chen et al. (1997)
Yeast(*S.cer.* BJ2407) hER(S)+β-gal(S)	Dexamethasone	50-02-2	neg.						Arnold et al. (1996)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Dexamethasone	50-02-2	pos.	13					Petit et al. (1999)
Yeast(*S.cer.* CYT10-5d) hER(S)+β-gal(S)	Dexamethasone	50-02-2	neg.	0					Chen et al. (1997)
MCF-7 hER(E)+CP	Diamyl phthalate	131-18-0				neg.			Soto et al. (1995)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Diamyl phthalate	131-18-0	neg.						Harris et al. (1997)
MCF-7 hER(E)+CP	Diazinon	333-41-5				neg.			Soto et al. (1995)
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	Dibenz[*a,c*]anthracene	215-58-7	neg.				neg.	102	Tran et al. (1996)
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	Dibenz[*a,c*]anthracene	215-58-7					neg.		Tran et al. (1996)
BG-1 hER(E)+Luc(S)	Dibenz[*a,h*]anthracene	53-70-3	pos.						Xenobiotic Detection Systems (2001)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Dibenz[*a,h*]anthracene	53-70-3	neg.		0				Clemons et al. (1998)
MCF-7 hER(E)+CP(F)	Dibenz[*a,h*]anthracene	53-70-3				neg.	pos.		Arcaro et al. (1999b)
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	Dibenz[*a,h*]anthracene	53-70-3	neg.				pos.	54	Tran et al. (1996)

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Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	Dibenz[*a,h*]anthracene	53-70-3					pos.	76	Tran et al. (1996)
T47D hER(E)+Luc(S)	3,3'-Dibromobisphenol A		pos.		0.000025				Meerts et al. (2001)
T47D hER(E)+Luc(S)	2,6-Dibromo-4-(2,4,6-tribromophenoxy)phenol		neg.				neg.		Meerts et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	2,4-Di-*tert* -butyl-6(5-chloro-2H-benzotriazol-2-yl)phenol	3864-99-1	neg.						Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	2,6-Di-*tert* -butyl-4-(dimethylamino-methyl)phenol	88-27-7	neg.						Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	4,6,-Di-*tert* -butyl-2,2'-ethylidene	35958-30-6	neg.						Miller et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	2,4-Dibutylphenol		neg.						Routledge and Sumpter (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	2,6-Dibutylphenol		neg.						Routledge and Sumpter (1997)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	2,6-Di-*tert* -butylphenol	128-39-2	pos.	<0.00000005					Miller et al. (2001)
CHO-K1 hERα(T)+Luc(T)†	Dibutyl phthalate	84-74-2	neg.		0				Otsuka Pharmaceutical (2001)
HeLa hERαdef(S)+Luc(S)	Dibutyl phthalate	84-74-2	neg.	0					Zacharewski et al. (1998)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Dibutyl phthalate	84-74-2		37					Zacharewski et al. (1998)
MCF-7 hER(E)+CP	Dibutyl phthalate	84-74-2				pos.			Harris et al. (1997)
MCF-7 hER(E)+CP	Dibutyl phthalate	84-74-2				pos.			Korner et al. (1998)
MCF-7 hER(E)+CP	Dibutyl phthalate	84-74-2				neg.			Soto et al. (1995)
MCF-7 hER(E)+Luc(T)+β-gal(T)	Dibutyl phthalate	84-74-2	pos.				neg.		Jobling et al. (1995)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Dibutyl phthalate	84-74-2	pos.	0.000000002	74				Vinggaard et al. (2000)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Dibutyl phthalate	84-74-2	pos.	0.00001					Harris et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Dibutyl phthalate	84-74-2	neg.	0					Coldham et al. (1997)

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Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Dibutyl phthalate	84-74-2	pos.	23.57					Petit et al. (1997)
ZR-75 hER(E)+CP	Dibutyl phthalate	84-74-2				pos.			Harris et al. (1997)
ZR-75 hER(E)+CP	Dibutyl phthalate	84-74-2				pos.			Jobling et al. (1995)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Dicamba	1918-00-9	pos.	17.74					Petit et al. (1997)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Dichlobenil	1194-65-6	pos.	18.13					Petit et al. (1997)
MCF-7 hER(E)+CP	2,5-Dichlorobiphenyl	34883-39-1				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP(F)	2,5-Dichlorobiphenyl	34883-39-1				pos.	neg.		Gierthy et al. (1997)
MCF-7 hER(E)+CP	2,6-Dichlorobiphenyl	33146-45-1				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP	3,5-Dichlorobiphenyl	34883-41-5				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP(F)	3,5-Dichlorobiphenyl	34883-41-5				pos.	neg.		Gierthy et al. (1997)
HeLa mER(T)+CAT(T)	2,5-Dichloro-2',3'-biphenyldiol		pos.	5.62					Garner et al. (1999)
HeLa mER(T)+CAT(T)	2,5-Dichloro-3',4'-biphenyldiol		pos.	18.62					Garner et al. (1999)
MCF-7 hER(E)+CP	2',5'-Dichloro-2-biphenylol	53905-30-9				pos.			Soto et al. (1995)
MCF-7 hER(E)+CP	2',5'-Dichloro-3-biphenylol	53905-29-6				pos.			Soto et al. (1995)
HeLa mER(T)+CAT(T)	2,5-Dichloro-4'-biphenylol	53905-28-5	pos.	20.31					Garner et al. (1999)
MCF-7 hER(E)+CP	2,5-Dichloro-4'-biphenylol	53905-28-5				pos.			Soto et al. (1995)
MCF-7 hER(E)+CP(F)	2,5-Dichloro-4'-biphenylol	53905-28-5				pos.	pos.		Gierthy et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	2,5-Dichloro-4'-biphenylol	53905-28-5	pos.	0.62					Coldham et al. (1997)
MCF-7 hER(E)+CP	3,5-Dichloro-2'-biphenylol					neg.			Soto et al. (1995)
MCF-7 hER(E)+CP	3,5-Dichloro-4'-biphenylol					neg.			Soto et al. (1995)
MCF-7 hER(E)+CP(F)	3,5-Dichloro-4'-diphenylol					pos.	pos.		Gierthy et al. (1997)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Dichlorophen	97-23-4	neg.						Miller et al. (2001)
MCF-7 hER(E)+CP	2,4-Dichlorophenol	120-83-2				neg.			Jones et al. (1998)

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ZR-75 hER(E)+CP	2,4-Dichlorophenol	120-83-2				neg.			Jobling et al. (1995)
MCF-7 hER(E)+CP	2,4-Dichlorophenoxyacetic acid	94-75-7				neg.			Soto et al. (1995)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	2,4-Dichlorophenoxyacetic acid	94-75-7	pos.	17.95					Petit et al. (1997)
MCF-7 hER(E)+CP	4-(2,4-Dichlorophenoxy)butanoic acid	94-82-6				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP	1,2-Dichloropropane	78-87-5				neg.			Soto et al. (1995)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Diclofop-methyl	51338-27-3	pos.	41.41					Petit et al. (1997)
CHO-K1 hERα(T)+Luc(T)†	Dicofol	115-32-2	neg.		0				Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Dicofol	115-32-2				neg.			Soto et al. (1995)
MCF-7(E3) hER(E)+CP	Dicofol	115-32-2				neg.			Vinggaard et al. (1999)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Dicofol	115-32-2	pos.	0.00001	3				Vinggaard et al. (1999)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Dicylcohexyl phthalate	84-61-7	neg.						Harris et al. (1997)
BG-1 hER(E)+Luc(S)	Dieldrin	60-57-1	pos.						Xenobiotic Detection Systems (2001)
ELT-3 hER(T)+Luc(T)+β-gal(T)	Dieldrin	60-57-1	pos.	16.87					Hodges et al. (2000)
MCF-7 hER(E)+CP	Dieldrin	60-57-1				neg.			Ramamoorthy et al. (1997a)
MCF-7 hER(E)+CP	Dieldrin	60-57-1				pos.			Soto et al. (1994)
MCF-7 hER(E)+CP	Dieldrin	60-57-1				pos.			Soto et al. (1995)
MCF-7 hER(E)+CP(F)	Dieldrin	60-57-1				neg.	neg.		Arcaro et al. (1998)
MCF-7 hER(T)+CAT(T)	Dieldrin	60-57-1		3.9					Ramamoorthy et al. (1997a)
T47D hER(E)+Luc(S)	Dieldrin	60-57-1	pos.	0.00024	24.49				Legler et al. (1999)
Yeast(*S.cer* .188R1) hER(S)+β-gal(S)	Dieldrin	60-57-1	pos.	0.000004	20		neg.		Graumann et al. (1999)
Yeast(*S.cer.* BJ2168) mER(S)+β-gal(S)	Dieldrin	60-57-1	pos.	6x (25 µM)*					Ramamoorthy et al. (1997a)

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Yeast(*S.cer.* BJ2168) mER(S)+β-gal(S)	Dieldrin	60-57-1	pos.	0.6x (100 µM)*					Ramamoorthy et al. (1997a)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Dieldrin	60-57-1	pos.	19.22					Petit et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Dienestrol	84-17-3	pos.	25.4					Coldham et al. (1997)
HEC-1 hERα(T)+CAT(T)+β-gal(T)	*cis,cis* - Diethyldihydroxytetrahydrochrysene			0.7	0.003				Sun et al. (1999)
HEC-1 hERα(T)+Luc(T)+β-gal(T)	*cis,cis* - Diethyldihydroxytetrahydrochrysene		pos.	0.04	0.05				Sun et al. (1999)
HEC-1 hERβ(T)+CAT(T)+β-gal(T)	*cis,cis* - Diethyldihydroxytetrahydrochrysene		neg.				pos.		Sun et al. (1999)
HEC-1 hERβ(T)+Luc(T)+β−gal(T)	*cis,cis* - Diethyldihydroxytetrahydrochrysene		neg.				pos.		Sun et al. (1999)
HEC-1 hERα(T)+CAT(T)+β-gal(T)	R,R-*cis,cis* - Diethyldihydroxytetrahydrochrysene			0.25	0.004				Sun et al. (1999)
HEC-1 hERβ(T)+CAT(T)+β-gal(T)	R,R-*cis,cis* - Diethyldihydroxytetrahydrochrysene		neg.				pos.		Sun et al. (1999)
HEC-1 hERα(T)+CAT(T)+β-gal(T)	S,S-*cis,cis* - Diethyldihydroxytetrahydrochrysene			2	0.001				Sun et al. (1999)
HEC-1 hERβ(T)+CAT(T)+β-gal(T)	S,S-*cis,cis* - Diethyldihydroxytetrahydrochrysene		pos.	0.025	0.4		pos.		Sun et al. (1999)
MCF-7 hER(E)+CP	Diethyl phthalate	84-66-2				neg.			Harris et al. (1997)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Diethyl phthalate	84-66-2	pos.	0.0000000005	384				Vinggaard et al. (2000)
ZR-75 hER(E)+CP	Diethyl phthalate	84-66-2				neg.			Harris et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Diethyl phthalate	84-66-2	pos.	0.00005					Harris et al. (1997)
BG-1 hER(E)+Luc(S)	Diethylstilbestrol	56-53-1	pos.	66.7	0.000015				Xenobiotic Detection Systems (2001)
BG-1 hER(E)+Luc(T)	Diethylstilbestrol	56-53-1	pos.	102					Rogers and Denison (2000)
CHO-K1 hERα(S)+Luc(S)†	Diethylstilbestrol	56-53-1	pos.		0.0000244				Otsuka Pharmaceutical (2001)
CHO-K1 hERα(T)+Luc(T)†	Diethylstilbestrol	56-53-1	pos.		0.0000189				Otsuka Pharmaceutical (2001)

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HEK293 hERα(T)+Luc(T)+β-gal(T)	Diethylstilbestrol	56-53-1	pos.	117					Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	Diethylstilbestrol	56-53-1	pos.	69					Kuiper et al. (1998)
HeLa hERα(T)+Luc(T)	Diethylstilbestrol	56-53-1	pos.	51					Sumida et al. (2001)
HeLa mER(T)+CAT(T)*	Diethylstilbestrol	56-53-1	pos.	119 (1 nM)					Shelby et al. (1996)
Ishikawa hER(E)+CP	Diethylstilbestrol	56-53-1				pos.			Le Guevel and Pakdel (2001)
MCF-7 hER(E)+CP	Diethylstilbestrol	56-53-1				pos.			Jones et al. (1998)
MCF-7 hER(E)+CP	Diethylstilbestrol	56-53-1				pos.			Morito et al. (2001a)
MCF-7 hER(E)+CP	Diethylstilbestrol	56-53-1				pos.			Soto et al. (1995)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Diethylstilbestrol	56-53-1	pos.	0.4	0.0005				Routledge and Sumpter (1997)
Yeast(*S.cer.* BJ2168) mER(S)+β-gal(S)	Diethylstilbestrol	56-53-1	pos.	100x (0.01 µM)*					Ramamoorthy et al. (1997a)
Yeast(*S.cer.* BJ2407) hER(S)+β-gal(S)	Diethylstilbestrol	56-53-1	pos.	1	0.0002				Arnold et al. (1996)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Diethylstilbestrol	56-53-1	pos.	74.3					Coldham et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Diethylstilbestrol	56-53-1	pos.	157	0.000353				Gaido et al. (1997)
Yeast(*S.cer.* BJ-ECZ) hER(S)+β-gal(S)	Diethylstilbestrol	56-53-1	pos.	25	0.0029				Le Guevel and Pakdel (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Diethylstilbestrol	56-53-1	pos.	120	0.0043				Le Guevel and Pakdel (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Diethylstilbestrol	56-53-1	pos.	100					Petit et al. (1999)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Diethylstilbestrol	56-53-1	pos.		0.0003				Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Diethylstilbestrol	56-53-1	pos.		0.0001				Morito et al. (2001a)
Yeast(*S.cer.* YRG-2) hERα(S)+β-gal(S)	Diethylstilbestrol	56-53-1	pos.	8x (0.1 µM)*					Lascombe et al. (2000)

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HEC-1 hERα(T)+CAT(T)+β-gal(T)	(*5R,11R*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol		pos.	63			neg.		Meyers et al. (1999)
HEC-1 hERβ(T)+CAT(T)+β-gal(T)	(*5R,11R*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol		neg.	0			pos.		Meyers et al. (1999)
HEC-1 hERα(T)+CAT(T)+β-gal(T)	(*5S,11S*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol		pos.	82			neg.		Meyers et al. (1999)
HEC-1 hERβ(T)+CAT(T)+β-gal(T)	(*5S,11S*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol		pos.	72			pos.		Meyers et al. (1999)
HeLa hERαdef(S)+Luc(S)	Dihexyl phthalate	84-75-3	neg.	0					Zacharewski et al. (1998)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Dihexyl phthalate	84-75-3		16					Zacharewski et al. (1998)
MCF-7 hER(E)+CP	Dihexyl phthalate	84-75-3				neg.			Harris et al. (1997)
ZR-75 hER(E)+CP	Dihexyl phthalate	84-75-3				neg.			Harris et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Dihexyl phthalate	84-75-3	neg.						Harris et al. (1997)
MCF-7 hER(E)+CP	Dihydrogenistein	21554-71-2				pos.			Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Dihydrogenistein	21554-71-2	pos.		2				Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Dihydrogenistein	21554-71-2	pos.		0.05				Morito et al. (2001a)
MCF-7 hER(E)+CP	Dihydroglycitein	94105-88-1				neg.			Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Dihydroglycitein	94105-88-1	pos.		10				Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Dihydroglycitein	94105-88-1	pos.		10				Morito et al. (2001a)
BG-1 hER(E)+Luc(T)	5α-Dihydrotestosterone	521-18-6	pos.	9					Rogers and Denison (2000)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	5α-Dihydrotestosterone	521-18-6	pos.	0.00025					Beresford et al. (2000)
Yeast(*S.cer.*) hER(S)+β-gal(S)	5α-Dihydrotestosterone	521-18-6	neg.						Routledge and Sumpter (1996)
Yeast(*S.cer* .939) hER(S)+β-gal(S)	5α-Dihydrotestosterone	521-18-6	pos.	0.034					Chen et al. (1997)

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Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	5α-Dihydrotestosterone	521-18-6	pos.	0.0005	0.431				Gaido et al. (1997)
HepG2 hERα(T)+Luc(T)+β-gal(T)	4,4'-Dihydroxybenzophenone	611-99-4	pos.	35			neg.	80	Gaido et al. (2000)
HepG2 hERβ(T)+Luc(T)+β-gal(T)	4,4'-Dihydroxybenzophenone	611-99-4	pos.	40			neg.	110µM	Gaido et al. (2000)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	4,4'-Dihydroxybenzophenone	611-94-4	pos.	0.000025					Miller et al. (2001)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	4,5-Dihydroxybenzo[*a*]pyrene	37571-88-3	[b]ni						Fertuck et al. (2001a)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	4,5-Dihydroxybenzo[*a*]pyrene	37571-88-3	ni						Fertuck et al. (2001a)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	7,8-Dihydroxybenzo[*a*]pyrene	60657-25-2	ni						Fertuck et al. (2001a)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	7,8-Dihydroxybenzo[*a*]pyrene	60657-25-2	ni						Fertuck et al. (2001a)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	9,10-Dihydroxybenzo[*a*]pyrene	58886-98-9	ni						Fertuck et al. (2001a)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	9,10-Dihydroxybenzo[*a*]pyrene	58886-98-9	ni						Fertuck et al. (2001a)
MCF-7 hER(E)+CP	4,4'-Dihydroxychalcone	3600-61-1				pos.			Miksicek (1993)
HepG2 hERα(T)+Luc(T)+β-gal(T)	Dihydroxy-DDE		pos.	100	0.074		neg.	100	Gaido et al. (2000)
HepG2 hERβ(T)+Luc(T)+β-gal(T)	Dihydroxy-DDE		pos.	25			pos.	30µM	Gaido et al. (2000)
MCF-7 hER(E)+CP	2,4'-Dihydroxy-4,6-dimethoxydihydrochalcone				2	pos.			Ichikawa et al. (1997)
MCF-7 hER(E)+CP	4,4'-Dihydroxy-2,6-dimethoxydihydrochalcone				0.6	pos.			Ichikawa et al. (1997)
HeLa hER(T)+CAT(T)	4'7-Dihydroxyflavone	2196-14-7	pos.	77					Miksicek (1993)
MCF-7(M) hER(E)+Luc(T)+β-gal(T)	4'7-Dihydroxyflavone	2196-14-7	pos.	45			pos.		Collins-Burow et al. (2000)
MCF-7 hER(E)+CP	Diisobutyl phthalate	84-69-5				pos.			Harris et al. (1997)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Diisobutyl phthalate	84-69-5	pos.	0.000000001	102				Vinggaard et al. (2000)

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Yeast(*S.cer.*) hER(S)+β-gal(S)	Diisobutyl phthalate	84-69-5	pos.	0.00001					Harris et al. (1997)
ZR-75 hER(E)+CP	Diisobutyl phthalate	84-69-5				pos.			Harris et al. (1997)
HeLa hERαdef(S)+Luc(S)	Diisodecyl phthalate	26761-40-0	neg.	0					Zacharewski et al. (1998)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Diisodecyl phthalate	26761-40-0		0					Zacharewski et al. (1998)
MCF-7 hER(E)+CP	Diisodecyl phthalate	26761-40-0				neg.			Harris et al. (1997)
ZR-75 hER(E)+CP	Diisodecyl phthalate	26761-40-0				neg.			Harris et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Diisodecyl phthalate	26761-40-0	neg.						Harris et al. (1997)
HeLa hERαdef(S)+Luc(S)	Diisoheptyl phthalate	41451-28-9	neg.	0					Zacharewski et al. (1998)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Diisoheptyl phthalate	41451-28-9		0					Zacharewski et al. (1998)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Diisohexyl phthalate	71850-09-4	neg.						Harris et al. (1997)
HeLa hERαdef(S)+Luc(S)	Diisononyl phthalate	28553-12-0	neg.	0					Zacharewski et al. (1998)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Diisononyl phthalate	28553-12-0		0					Zacharewski et al. (1998)
MCF-7 hER(E)+CP	Diisononyl phthalate	28553-12-0				pos.			Harris et al. (1997)
ZR-75 hER(E)+CP	Diisononyl phthalate	28553-12-0				pos.			Harris et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Diisononyl phthalate	28553-12-0	weak						Harris et al. (1997)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	2,6-Diisopropylnaphthalene	24157-81-1	pos.	0.000000003	53				Vinggaard et al. (2000)
HepG2 hERα(T)+Luc(T)+β-gal(T)	4,4'-Dimethoxybenzhydrol	728-87-0	weak	10			neg.	95	Gaido et al. (2000)
HepG2 hERβ(T)+Luc(T)+β-gal(T)	4,4'-Dimethoxybenzhydrol	728-87-0	pos.	10			neg.	100µM	Gaido et al. (2000)
HepG2 hERα(T)+Luc(T)+β-gal(T)	Dimethoxy-DDE		pos.	30			neg.	95	Gaido et al. (2000)

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HepG2 hERβ(T)+Luc(T)+β-gal(T)	Dimethoxy-DDE		pos.	15			neg.	80µM	Gaido et al. (2000)
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	7,12-Dimethylbenz[*a*]anthracene	57-97-6	neg.				neg.	102	Tran et al. (1996)
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	7,12-Dimethylbenz[*a*]anthracene	57-97-6					neg.		Tran et al. (1996)
MCF-7 hER(E)+CP	Dimethyl isophthalate	1459-93-4				neg.			Soto et al. (1995)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Dimethyl isophthalate	1459-93-4	neg.						Harris et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Dimethyl phthalate	131-11-3	neg.						Harris et al. (1997)
HeLa hERαdef(S)+Luc(S)	Dimethyl sulfoxide	67-68-5							Connor et al. (1997)
HeLa hERα(T)+Luc(T)	Dimethyl sulfoxide	67-68-5	neg.	11.1					Sumida et al. (2001)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Dimethyl sulfoxide	67-68-5	pos.	0.03					Charles et al. (2000b)
MCF-7 hER(E)+CP	Dimethyl sulfoxide	67-68-5							Ramamoorthy et al. (1997a)
MCF-7 hER(E)+CP(F)	Dimethyl sulfoxide	67-68-5				neg.	neg.		Arcaro et al. (1999b)
MCF-7 hER(E)+Luc(T)	Dimethyl sulfoxide	67-68-5		0					Sumida et al. (2001)
MCF-7 hER(T)+CAT(T)	Dimethyl sulfoxide	67-68-5	pos.	100					Connor et al. (1997)
MCF-7 hER(T)+CAT(T)	Dimethyl sulfoxide	67-68-5		3.9					Ramamoorthy et al. (1997a)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Dimethyl sulfoxide	67-68-5	pos.	17.94					Petit et al. (1997)
MCF-7 hER(E)+CP	Dimethylterephthalate	120-61-6				neg.			Soto et al. (1995)
HEC-1 hERα(T)+CAT(T)+β-gal(T)	(*5R,11R*)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol		pos.	75			neg.		Meyers et al. (1999)
HEC-1 hERβ(T)+CAT(T)+β-gal(T)	(*5R,11R*)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol		neg.	11			pos.		Meyers et al. (1999)
HEC-1 hERα(T)+CAT(T)+β-gal(T)	(*5S,11S*)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol		pos.	88			neg.		Meyers et al. (1999)

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HEC-1 hERβ(T)+CAT(T)+β-gal(T)	(*5S,11S*)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol		pos.	86			neg.		Meyers et al. (1999)
HEC-1 hERα(T)+CAT(T)+β-gal(T)	5,11-*trans* -Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol		pos.	93			neg.		Meyers et al. (1999)
HEC-1 hERβ(T)+CAT(T)+β-gal(T)	5,11-*trans* -Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol		pos.	80			neg.		Meyers et al. (1999)
Yeast(*S.cer.*) hER(S)+β-gal(S)	2,4-Dinonylphenol	137-99-5	pos.	0.000002	100				Routledge and Sumpter (1997)
MCF-7 hER(E)+CP	Dinonyl phthalate	84-76-4				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP	Dinoseb	88-85-7				neg.			Soto et al. (1995)
HeLa hERαdef(S)+Luc(S)	Di-*n* -octyl phthalate	117-84-0	neg.	0					Zacharewski et al. (1998)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Di-*n* -octyl phthalate	117-84-0		0					Zacharewski et al. (1998)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Di-*n* -octyl phthalate	117-84-0	neg.						Harris et al. (1997)
HeLa hER(T)+CAT(T)	Diosgenin	512-04-9	neg.	21.25					Miksicek (1994)
BG-1 hER(E)+Luc(S)	1,4-Dioxane	123-91-1	neg.						Xenobiotic Detection Systems (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Diphenyl phthalate	84-62-8	neg.						Harris et al. (1997)
HEC-1 hERβ(T)+CAT(T)+β-gal(T)	5,11-*trans* -Dipropyl-5,6,11,12-tetrahydrochrysene-2,8 diol		pos.	63			neg.		Meyers et al. (1999)
HEC-1 hERα(T)+CAT(T)+β-gal(T)	5,11-*trans* -Dipropyl-5,6,11,12-tetrahydrochrysene-2,8 diol		pos.	45			neg.		Meyers et al. (1999)
HEC-1 hERα(T)+CAT(T)+β-gal(T)	(*5R,11R*)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8 diol		pos.	70			neg.		Meyers et al. (1999)
HEC-1 hERβ(T)+CAT(T)+β-gal(T)	(*5R,11R*)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8 diol		neg.	0			pos.		Meyers et al. (1999)
HEC-1 hERα(T)+CAT(T)+β-gal(T)	(*5S,11S*)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8 diol		pos.	76			neg.		Meyers et al. (1999)
HEC-1 hERβ(T)+CAT(T)+β-gal(T)	(*5S,11S*)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8 diol		neg.	10			pos.		Meyers et al. (1999)
BG-1 hER(E)+Luc(S)	Disulfoton	298-04-4	neg.						Xenobiotic Detection Systems (2001)

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MCF-7 hER(E)+CP	Ditridecyl phthalate	119-06-2				neg.			Harris et al. (1997)
ZR-75 hER(E)+CP	Ditridecyl phthalate	119-06-2				neg.			Harris et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Ditridecyl phthalate	119-06-2	neg.						Harris et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Diundecyl phthalate	3648-20-2	neg.						Harris et al. (1997)
MCF-7(E3) hER(E)+CP	Diuron	330-54-1				neg.			Vinggaard et al. (1999)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Diuron	330-54-1	pos.	17.08					Petit et al. (1997)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Dodecyl gallate	1166-52-5	neg.						Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Dodecylparaben	2664-60-0	neg.						Miller et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-*sec* -Dodecylphenol		pos.	0.0000001	2000				Routledge and Sumpter (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-*sec* -Dodecylphenol dipropoxylate		neg.	0					Routledge and Sumpter (1997)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Dodemorph	1593-77-7	pos.	16.13					Petit et al. (1997)
COS-1 mERα(T)+Lucb(T)	Droloxifene	82413-20-5	pos.				pos.		Tremblay et al. (1998)
COS-1 mERα(T)+Luctk(T)	Droloxifene	82413-20-5	neg.				pos.		Tremblay et al. (1998)
COS-1 mERβ(T)+Lucb(T)	Droloxifene	82413-20-5	neg.				pos.		Tremblay et al. (1998)
COS-1 mERβ(T)+Luctk(T)	Droloxifene	82413-20-5	neg.				pos.		Tremblay et al. (1998)
COS-1 mERα(T)+Lucb(T)	EM-652	37607-02-6	neg.				pos.		Tremblay et al. (1998)
COS-1 mERα(T)+Luctk(T)	EM-652	37607-02-6	neg.				pos.		Tremblay et al. (1998)
COS-1 mERβ(T)+Lucb(T)	EM-652	37607-02-6	neg.				pos.		Tremblay et al. (1998)
COS-1 mERβ(T)+Luctk(T)	EM-652	37607-02-6	neg.				pos.		Tremblay et al. (1998)
BG-1 hER(E)+Luc(S)	α−Endosulfan	959-98-8	pos.						Xenobiotic Detection Systems (2001)
ELT-3 hER(T)+Luc(T)+β-gal(T)	α−Endosulfan	959-98-8	pos.	6.7					Hodges et al. (2000)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
MCF-7 hER(E)+CP	α−Endosulfan	959-98-8				pos.			Soto et al. (1994)
MCF-7 hER(E)+CP	α−Endosulfan	959-98-8				pos.			Soto et al. (1995)
MCF-7 hER(E)+CP(F)	α−Endosulfan	959-98-8			10		neg.		Arcaro et al. (1998)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	α−Endosulfan	959-98-8	pos.	25.87					Petit et al. (1997)
HEK293 hERα(T)+Luc(T)+β-gal(T)	α,β-Endosulfan	115-29-7	pos.	6					Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	α,β-Endosulfan	115-29-7	neg.	1					Kuiper et al. (1998)
HeLa mER(T)+CAT(T)*	α,β-Endosulfan	115-29-7	pos.	6 (1 µM)					Shelby et al. (1996)
MCF-7 hER(E)+CP	α,β-Endosulfan	115-29-7				pos.			Soto et al. (1994)
MCF-7 hER(E)+CP	α,β-Endosulfan	115-29-7				pos.			Soto et al. (1995)
T47D hER(E)+Luc(S)	α,β-Endosulfan	115-29-7	pos.	0.001	5.92				Legler et al. (1999)
Yeast(*S.cer* .188R1) hER(S)+β-gal(S)	α,β-Endosulfan	115-29-7	pos.	0.000004	20		neg.		Graumann et al. (1999)
Yeast(*S.cer.* BJ2168) mER(S)+β-gal(S)	α,β-Endosulfan	115-29-7	pos.	16x (25 µM)*					Ramamoorthy et al. (1997a)
Yeast(*S.cer.* BJ2168) mER(S)+β-gal(S)	α,β-Endosulfan	115-29-7	pos.	33x (100 µM)*					Ramamoorthy et al. (1997a)
BG-1 hER(E)+Luc(S)	β−Endosulfan	33213-65-9	pos.						Xenobiotic Detection Systems (2001)
ELT-3 hER(T)+Luc(T)+β-gal(T)	β−Endosulfan	33213-65-9	pos.	6.7					Hodges et al. (2000)
MCF-7 hER(E)+CP	β−Endosulfan	33213-65-9				pos.			Soto et al. (1994)
MCF-7 hER(E)+CP	β−Endosulfan	33213-65-9				pos.			Soto et al. (1995)
MCF-7 hER(E)+CP(F)	β−Endosulfan	33213-65-9			10		neg.		Arcaro et al. (1998)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	β−Endosulfan	33213-65-9	pos.	20.72					Petit et al. (1997)
BG-1 hER(E)+Luc(S)	Endrin	72-20-8	pos.						Xenobiotic Detection Systems (2001)
BG-1 hER(E)+Luc(S)	Endrin	72-20-8	pos.						Xenobiotic Detection Systems (2001)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
BG-1 hER(E)+Luc(S)	Endrin aldehyde	7421-93-4	neg.						Xenobiotic Detection Systems (2001)
BG-1 hER(E)+Luc(S)	Epichlorohydrin	106-89-8	neg.						Xenobiotic Detection Systems (2001)
MCF-7 hER(E)+CP	Epidermal growth factor	62229-50-9				weak			Jones et al. (1998)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	17α-Epitestosterone	481-30-1	neg.						Le Guevel and Pakdel (2001)
MCF-7 hER(E)+CP	d-Equilenin	517-09-9				pos.			Soto et al. (1995)
CHO-K1 hERα(T)+Luc(T)†	Equilin	474-86-2	pos.		0.0000403				Otsuka Pharmaceutical (2001)
CHO-K1 hERα(S)+Luc(S)†	Equol	531-95-3	pos.		0.04				Otsuka Pharmaceutical (2001)
CHO-K1 hERα(T)+Luc(T)†	Equol	531-95-3	pos.		0.0134				Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Equol	531-95-3				pos.			Morito et al. (2001a)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Equol	531-95-3	pos.	0.085					Coldham et al. (1997)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Equol	531-95-3	pos.	86.28					Petit et al. (1997)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Equol	531-95-3	pos.		0.4				Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Equol	531-95-3	pos.		0.02				Morito et al. (2001a)
CHO-K1 hERα(T)+Luc(T)†	17α-Estradiol	57-91-0	pos.		0.0000456				Otsuka Pharmaceutical (2001)
Ishikawa hER(E)+CP	17α-Estradiol	57-91-0				pos.			Le Guevel and Pakdel (2001)
MCF-7 hER(E)+CP	17α-Estradiol	57-91-0				pos.			Soto et al. (1995)
T47D hER(E)+Luc(T)	17α-Estradiol	57-91-0	pos.	3					Hoogenboom et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	17α-Estradiol	57-91-0	pos.	0.015	0.002				Routledge and Sumpter (1996)
Yeast(*S.cer.*) hER(S)+β-gal(S)	17α-Estradiol	57-91-0	pos.	0.05	0.004				Routledge and Sumpter (1997)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	17α-Estradiol	57-91-0	pos.	5.25					Coldham et al. (1997)
Yeast(*S.cer.* BJ-ECZ) hER(S)+β-gal(S)	17α-Estradiol	57-91-0	pos.	14	0.0052				Le Guevel and Pakdel (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	17α-Estradiol	57-91-0	pos.	4.4	0.14				Le Guevel and Pakdel (2001)
BG-1 hER(E)+Luc(S)	17β-Estradiol	50-28-2	pos.	100	0.00001				Xenobiotic Detection Systems (2001)
BG-1 hER(E)+Luc(T)	17β-Estradiol	50-28-2	pos.	100					Rogers and Denison (2000)
CHO-K1 hERα(T)+Luc(T)†	17β-Estradiol	50-28-2	pos.		0.0000123				Otsuka Pharmaceutical (2001)
COS-1 mERα(T)+Lucb(T)	17β-Estradiol	50-28-2	pos.						Tremblay et al. (1998)
COS-1 mERα(T)+Luctk(T)	17β-Estradiol	50-28-2	pos.						Tremblay et al. (1998)
COS-1 mERβ(T)+Lucb(T)	17β-Estradiol	50-28-2	pos.						Tremblay et al. (1998)
COS-1 mERβ(T)+Luctk(T)	17β-Estradiol	50-28-2	pos.						Tremblay et al. (1998)
ELT-3 hER(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	100					Hodges et al. (2000)
HEC-1 hERα(T)+CAT(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.		0.000015				Kraichely et al. (2000)
HEC-1 hERα(T)+CAT(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	100					Meyers et al. (1999)
HEC-1 hERα(T)+CAT(T)+β-gal(T)	17β-Estradiol	50-28-2		100	0.00002				Sun et al. (1999)
HEC-1 hERα(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	100	0.00002				Sun et al. (1999)
HEC-1 hERβ(T)+CAT(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.		0.0001				Kraichely et al. (2000)
HEC-1 hERβ(T)+CAT(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	100					Meyers et al. (1999)
HEC-1 hERβ(T)+CAT(T)+β-gal(T)	17β-Estradiol	50-28-2		100	0.0001				Sun et al. (1999)
HEC-1 hERβ(T)+Luc(T)+β−gal(T)	17β-Estradiol	50-28-2	pos.						Sun et al. (1999)

Sorted by Substance and Assay

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
HEK293+hERα(S)+Luc(S)	17β-Estradiol	50-28-2	pos.						Meerts et al. (2001)
HEK293+hERα(S)+Luc(S)	17β-Estradiol	50-28-2	pos.						Meerts et al. (2001)
HEK293 hERα(S)+Luc(T)	17β-Estradiol	50-28-2	pos.						Seinen et al. (1999)
HEK-293 hERα(T)+Luc(T)	17β-Estradiol	50-28-2	pos.	100			pos.	100	Collins-Burow et al. (2000)
HEK-293 hERα(T)+Luc(T)	17β-Estradiol	50-28-2	pos.	100				100	Collins-Burow et al. (2000)
HEK293 hERα(T)+Luc(T)	17β-Estradiol	50-28-2	pos.						Seinen et al. (1999)
HEK293 hERα(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	100					Kuiper et al. (1998)
HEK293 hERβ(S)+Luc(T)	17β-Estradiol	50-28-2	pos.						Seinen et al. (1999)
HEK293 hERβ(T)+Luc(T)	17β-Estradiol	50-28-2	pos.						Seinen et al. (1999)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	100					Kuiper et al. (1998)
HeLa hERαdef(S)+Luc(S)	17β-Estradiol	50-28-2		100					Connor et al. (1997)
HeLa hERαdef(S)+Luc(S)	17β-Estradiol	50-28-2	pos.	100					Moore et al. (1997)
HeLa hERαdef(S)+Luc(S)	17β-Estradiol	50-28-2	pos.	100					Zacharewski et al. (1998)
HeLa hERα(T)+Luc(T)	17β-Estradiol	50-28-2	pos.	100					Sumida et al. (2001)
HeLa hERα(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	100	0.00001				Gaido et al. (1999)
HeLa hERβ(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	100	0.000005				Gaido et al. (1999)
HeLa hER(S)+Luc(S)	17β-Estradiol	50-28-2	pos.		0.00003				Balaguer et al. (1996)
HeLa hER(T)+CAT(T)	17β-Estradiol	50-28-2	pos.	100					Miksicek (1993)
HeLa hER(T)+CAT(T)	17β-Estradiol	50-28-2	pos.	100	0.00001				Miksicek (1994)
HeLa mER(T)+CAT(T)	17β-Estradiol	50-28-2	pos.	100					Garner et al. (1999)
HeLa mER(T)+CAT(T)	17β-Estradiol	50-28-2		100		pos.			Makela et al. (1994)
HeLa mER(T)+CAT(T)*	17β-Estradiol	50-28-2	pos.	54x (1 nM)					Makela et al. (1994)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
HeLa mER(T)+CAT(T)*	17β-Estradiol	50-28-2	pos.	100 (1 nM)					Shelby et al. (1996)
HepG2 hERα(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	100	0.004				Gaido et al. (1999)
HepG2 hERα(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	100	0.003		neg.	100	Gaido et al. (2000)
HepG2 hERα(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	100	0.0099				Gould et al. (1998)
HepG2 hERβ(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	100	0.008				Gaido et al. (1999)
HepG2 hERβ(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	100	0.007		neg.	100µM	Gaido et al. (2000)
HepG2 hER(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	100					Ramamoorthy et al. (1997b)
HepG2 rERα(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	100	0.001				Gaido et al. (1999)
Ishikawa hER(E)+CP	17β-Estradiol	50-28-2				pos.			Le Guevel and Pakdel (2001)
Ishikawa hER(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	1				100%	Klotz et al. (1997)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	100	0.000096				Charles et al. (2000a)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	1					Charles et al. (2000b)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.		0.00058				Clemons et al. (1998)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	100					Connor et al. (1996)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2		100	0.00035				Fertuck et al. (2001a)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	**1**	0.00035				Fertuck et al. (2001b)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2		100					Fielden et al. (1997)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2		**1**	0.000053				Matthews et al. (2001)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2		100					Zacharewski et al. (1998)
MCF-7 hERβdef(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2		**1**	0.000083				Matthews et al. (2001)
MCF-7 hER(E)+CAT(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.					100	Bonefeld-Jørgensen et al. (2001)
MCF-7 hER(E)+CP	17β-Estradiol	50-28-2				pos.			Dodge et al. (1996)
MCF-7 hER(E)+CP	17β-Estradiol	50-28-2				pos.			Fielden et al. (1997)
MCF-7 hER(E)+CP	17β-Estradiol	50-28-2				pos.			Go et al. (1999)
MCF-7 hER(E)+CP	17β-Estradiol	50-28-2				pos.			Harris et al. (1997)
MCF-7 hER(E)+CP	17β-Estradiol	50-28-2				pos.	pos.		Jones et al. (1998)
MCF-7 hER(E)+CP	17β-Estradiol	50-28-2			0.00001	pos.			Korner et al. (1998)
MCF-7 hER(E)+CP	17β-Estradiol	50-28-2				pos.			Makela et al. (1994)
MCF-7 hER(E)+CP	17β-Estradiol	50-28-2				pos.			Mellanen et al. (1996)
MCF-7 hER(E)+CP	17β-Estradiol	50-28-2				pos.			Miksicek (1993)
MCF-7 hER(E)+CP	17β-Estradiol	50-28-2				pos.			Miodini et al. (1999)
MCF-7 hER(E)+CP	17β-Estradiol	50-28-2				pos.			Moore et al. (1997)
MCF-7 hER(E)+CP	17β-Estradiol	50-28-2				pos.			Morito et al. (2001a)
MCF-7 hER(E)+CP	17β-Estradiol	50-28-2				pos.			Nakagawa and Suzuki (2001)
MCF-7 hER(E)+CP	17β-Estradiol	50-28-2				pos.			Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	17β-Estradiol	50-28-2				pos.			Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	17β-Estradiol	50-28-2				pos.			Ramamoorthy et al. (1997a)
MCF-7 hER(E)+CP	17β-Estradiol	50-28-2				pos.			Soto et al. (1994)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
MCF-7 hER(E)+CP	17β-Estradiol	50-28-2				pos.			Soto et al. (1995)
MCF-7 hER(E)+CP	17β-Estradiol	50-28-2				pos.			Bonefeld-Jørgensen et al. (2001)
MCF-7 hER(E)+CP(F)	17β-Estradiol	50-28-2			0.00001	pos.			Arcaro et al. (1999a)
MCF-7 hER(E)+CP(F)	17β-Estradiol	50-28-2			0.0003	pos.			Arcaro et al. (1999b)
MCF-7 hER(E)+CP(F)	17β-Estradiol	50-28-2				pos.	neg.		Gierthy et al. (1997)
MCF-7 hER(E)+CP(F)	17β-Estradiol	50-28-2				pos.			Tamir et al. (2000)
MCF-7 hER(E)+Luc(S)	17β-Estradiol	50-28-2			0.0015				Kramer et al. (1997)
MCF-7 hER(E)+Luc(T)	17β-Estradiol	50-28-2	pos.						Yoshihara et al. (2001)
MCF-7(M) hER(E)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.	100					Collins-Burow et al. (2000)
MCF-7 hER(E)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2	pos.						Jobling et al. (1995)
MCF-7 hER(E)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2		**1**					Klotz et al. (1996)
MCF-7 hER(E)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2		**1**				100%	Klotz et al. (1997)
MCF-7 hER(T)+CAT(T)	17β-Estradiol	50-28-2	pos.	312			neg.	100	Connor et al. (1997)
MCF-7 hER(T)+CAT(T)	17β-Estradiol	50-28-2		100					Ramamoorthy et al. (1997a)
MCF-7 hER(T)+CAT(T)	17β-Estradiol	50-28-2		100					Ramamoorthy et al. (1997b)
MCF-7 hER(T)+Luc(T)	17β-Estradiol	50-28-2		100					Ramamoorthy et al. (1997b)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2		100	0.00013				Fertuck et al. (2001a)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	17β-Estradiol	50-28-2		**1**	0.00013				Fertuck et al. (2001b)
MCF-7(M) hER(E)+CP	17β-Estradiol	50-28-2				pos.			Collins-Burow et al. (2000)
MCF-7(Bos) hER(E)+CP	17β-Estradiol	50-28-2		100	0.00000122	pos.			Schlumpf et al. (2001)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
MCF-7(BUS) hER(E)+CP	17β-Estradiol	50-28-2				pos.			Schafer et al. (1999)
MCF-7(E3) hER(E)+CP	17β-Estradiol	50-28-2			0.000008	pos.			Vinggaard et al. (1999)
MCF-7(E3) hER(E)+CP	17β-Estradiol	50-28-2			0.00152	pos.			Wiese et al. (1997)
MCF-7(McGrath) hER(E)+CP	17β-Estradiol	50-28-2				pos.			Nakagawa and Suzuki (2001)
MCF-7(MELN41) hER(E)+Luc(S)	17β-Estradiol	50-28-2	pos.		0.0001				Lascombe et al. (2000)
MDA-MB-231 hERα(T)+Luc(T)	17β-Estradiol	50-28-2	pos.						Bonefeld-Jørgensen et al. (2001)
MDA-MB-231 hER(T)+Luc(T)	17β-Estradiol	50-28-2	pos.	1					Ramamoorthy et al. (1997b)
T47D hER(E)+CAT(T)	17β-Estradiol	50-28-2	pos.	1					Nakagawa and Suzuki (2001)
T47D hER(E)+CP	17β-Estradiol	50-28-2				pos.			Mellanen et al. (1996)
T47D hER(E)+CP	17β-Estradiol	50-28-2				pos.			Schafer et al. (1999)
T47D hER(E)+CP	17β-Estradiol	50-28-2				pos.			Tamir et al. (2000)
T47D hER(E)+Luc(S)	17β-Estradiol	50-28-2	pos.	100	0.000006				Legler et al. (1999)
T47D hER(E)+Luc(S)	17β-Estradiol	50-28-2			0.00001				Meerts et al. (2001)
T47D hER(E)+Luc(T)	17β-Estradiol	50-28-2	pos.	100					Hoogenboom et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	1					Beresford et al. (2000)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.		0.00011-0.00056				De Boever et al. (2001)
Yeast(*S.cer* .) hERα(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	1	0.000051				Elsby et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	1	0.0002				Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	1	0.00004				Vinggaard et al. (1999)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	1	0.00002				Vinggaard et al. (2000)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
Yeast(*S.cer.*) hERα(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.						Yoshihara et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)+S9	17β-Estradiol	50-28-2	pos.						Yoshihara et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	1					Harris et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	1	0.0006				Moffat et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	1	0.00015				Odum et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	1	0.00013				Rajapakse et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	1	0.00003				Routledge and Sumpter (1996)
Yeast(*S.cer.*) hER(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	1	0.0002				Routledge and Sumpter (1997)
Yeast(*S.cer* .188R1) hER(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	1	0.00007				Graumann et al. (1999)
Yeast(*S.cer* .939) hER(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	1				100	Chen et al. (1997)
Yeast(*S.cer.* BJ2168) mER(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	100x (0.01 uM)*					Ramamoorthy et al. (1997a)
Yeast(*S.cer.* BJ2407) hER(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	1	0.0002				Arnold et al. (1996)
Yeast(*S.cer.* BJ2407) hER(S)+β-gal(S)	17β-Estradiol	50-28-2		100x (0.01 µM)*					Klotz et al. (1996)
Yeast(*S.cer.* BJ3503) hER(S)+β-gal(S)	17β-Estradiol	50-28-2		1	0.00009				Ramamoorthy et al. (1997b)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	100					Coldham et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	100	0.000225				Gaido et al. (1997)
Yeast(*S.cer.* BJ-ECZ) hER(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	100	0.00074				Le Guevel and Pakdel (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	100	0.0052				Le Guevel and Pakdel (2001)

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Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	100					Petit et al. (1997)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	100					Petit et al. (1999)
Yeast(*S.cer.* CYT10-5d) hER(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	100x (0.1 µM)*			pos.	100	Chen et al. (1997)
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.	1			neg.	100	Tran et al. (1996)
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	17β-Estradiol	50-28-2						100	Tran et al. (1996)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.		0.00002				Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.		0.00025				Morito et al. (2001b)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.		0.00002				Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.		0.0002				Morito et al. (2001b)
Yeast(*S.cer.* YRG-2) hERα(S)+β-gal(S)	17β-Estradiol	50-28-2	pos.						Lascombe et al. (2000)
ZR-75 hER(E)+CP	17β-Estradiol	50-28-2				pos.			Harris et al. (1997)
ZR-75 hER(E)+CP	17β-Estradiol	50-28-2				pos.			Jobling et al. (1995)
ZR-75 hER(E)+CP	17β-Estradiol	50-28-2							Nakagawa and Suzuki (2001)
ZR-75-1 hER(E)+CP	17β-Estradiol	50-28-2				pos.			Schafer et al. (1999)
T47D hER(E)+Luc(T)	Estradiol benzoate	50-50-0	pos.	25					Hoogenboom et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Estradiol benzoate	50-50-0	pos.	0.08	0.0018				Odum et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	17β-Estradiol-3β-*D* -gluconate	15270-30-1	neg.						Routledge and Sumpter (1996)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	17β-Estradiol-3β-*D* -gluconate	15270-30-1	pos.	0.32					Coldham et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	17β-Estradiol glucuronide	1806-98-0	neg.	0					Routledge and Sumpter (1997)

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Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	17β-Estradiol-3-glucuronide-17-sulfate		neg.	0					Coldham et al. (1997)
T47D hER(E)+Luc(T)	17β-Estradiol oleate	82204-99-7	pos.	1					Hoogenboom et al. (2001)
T47D hER(E)+Luc(T)	17β-Estradiol palmitate	126143-99-5	pos.	5					Hoogenboom et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	17β-Estradiol-3-sulfate	28814-94-0	pos.	0.003					Beresford et al. (2000)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	17β-Estradiol-3-sulfate	28814-94-0	pos.	0.00003					Beresford et al. (2000)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	17β-Estradiol-3-sulfate	28814-94-0	pos.	0.1					Beresford et al. (2000)
Yeast(*S.cer.*) hER(S)+β-gal(S)	17β-Estradiol-3-sulfate	28814-94-0	pos.	0.15	0.02				Routledge and Sumpter (1996)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	17β-Estradiol-3-sulfate	28814-94-0	pos.	0.01					Coldham et al. (1997)
MCF-7(E3) hER(E)+CP	6,8-Estrapentaene-3,17β-diol	1423-97-8			0.0125	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	6-Estratetraene-3,17β-diol	7291-41-0			0.00419	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	7-Estratetraene-3,17β-diol	3563-27-7			0.00154	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	9-Estratetraene-3,17β-diol	791-69-5			0.099	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	Estratriene-1,17β-diol	126654-96-4			1.59	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	Estratriene-2,17β-diol	2259-89-4			0.033	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	Estratriene-3,16α-diol	1090-04-6			0.237	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	Estratriene-3,17α-diol				0.382	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	Estratriene-4,17β-diol	17592-89-1			4.76	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	Estratriene-3,11α,17β-triol	1464-61-5			0.0365	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	Estratriene-3,11β,17β-triol	5444-22-4			0.0163	pos.			Wiese et al. (1997)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Estratriene-3,6α,17β-triol	1229-24-9	pos.	3.2	0.003				Charles et al. (2000a)
MCF-7(E3) hER(E)+CP	Estratriene-3,6α,17β-triol	1229-24-9			0.186	pos.			Wiese et al. (1997)

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MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Estratriene-3,6β,17β-triol	3583-03-7	pos.	0.32	0.03				Charles et al. (2000a)
MCF-7(E3) hER(E)+CP	Estratriene-3,6β,17β-triol	3583-03-7			0.0571	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	Estratriene-3,7α,17β-triol	3398-11-6			0.153	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	Estratriene-3,7β,17β-triol	2487-46-9			0.0122	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	Estratrien-17β-ol	2529-64-8			0.316	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	Estratrien-3-ol	53-63-4			0.118	pos.			Wiese et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Estratrien-3-ol	53-63-4	pos.	0.05	0.0032				Odum et al. (1997)
MCF-7 hER(E)+CP	Estriol	50-27-1				pos.			Soto et al. (1995)
MCF-7(E3) hER(E)+CP	Estriol	50-27-1			0.0483	pos.			Wiese et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Estriol	50-27-1	pos.	0.025	0.008				Routledge and Sumpter (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Estriol	50-27-1	pos.	0.63					Coldham et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Estriol	50-27-1	pos.	0.004	0.0616				Gaido et al. (1997)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Estriol	50-27-1	pos.	13.5	0.00071				Charles et al. (2000a)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Estriol	50-27-1	pos.	75					Petit et al. (1999)
CHO-K1 hERα(T)+Luc(T)†	Estrone	53-16-7	pos.		0.0000193				Otsuka Pharmaceutical (2001)
Ishikawa hER(E)+CP	Estrone	53-16-7				pos.			Le Guevel and Pakdel (2001)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Estrone	53-16-7	pos.	1.5	0.0063				Charles et al. (2000a)
MCF-7 hER(E)+CP	Estrone	53-16-7				pos.			Makela et al. (1994)
MCF-7 hER(E)+CP	Estrone	53-16-7				pos.			Soto et al. (1995)
MCF-7(E3) hER(E)+CP	Estrone	53-16-7			0.0772	pos.			Wiese et al. (1997)

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T47D hER(E)+CP	Estrone	53-16-7				pos.			Makela et al. (1994)
T47D hER(E)+Luc(T)	Estrone	53-16-7	pos.	100					Hoogenboom et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Estrone	53-16-7	pos.	0.67	0.0003				Routledge and Sumpter (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Estrone	53-16-7	pos.	9.6					Coldham et al. (1997)
Yeast(*S.cer.* BJ-ECZ) hER(S)+β-gal(S)	Estrone	53-16-7	pos.	35	0.0021				Le Guevel and Pakdel (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Estrone	53-16-7	pos.	19	0.022				Le Guevel and Pakdel (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Estrone	53-16-7	pos.	74					Petit et al. (1999)
HeLa hER(T)+CAT(T)	Ethanol	64-17-5	pos.	26					Miksicek (1993)
MCF-7 hER(E)+CP	Ethanol	64-17-5				neg.			Go et al. (1999)
MCF-7 hER(E)+CP	Ethanol	64-17-5				neg.			Harris et al. (1997)
MCF-7 hER(E)+CP	Ethanol	64-17-5				neg.			Jones et al. (1998)
T47D hER(E)+Luc(S)	Ethanol	64-17-5	neg.						Meerts et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Ethanol	64-17-5	neg.	0	0				Vinggaard et al. (2000)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Ethanol	64-17-5	neg.						Harris et al. (1997)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Ethanol	64-17-5	pos.	14					Petit et al. (1997)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Ethanol	64-17-5	pos.	11					Petit et al. (1999)
ZR-75 hER(E)+CP	Ethanol	64-17-5				neg.			Harris et al. (1997)
CHO-K1 hERα(S)+Luc(S)†	17α-Ethinyl estradiol	57-63-6	pos.		0.0000073				Otsuka Pharmaceutical (2001)
CHO-K1 hERα(T)+Luc(T)†	17α-Ethinyl estradiol	57-63-6	pos.		0.0000144				Otsuka Pharmaceutical (2001)
Ishikawa hER(E)+CP	17α-Ethinyl estradiol	57-63-6				pos.			Le Guevel and Pakdel (2001)
MCF-7 hER(E)+CP	17α-Ethinyl estradiol	57-63-6				pos.			Soto et al. (1995)

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Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	17α-Ethinyl estradiol	57-63-6	pos.	88.8					Coldham et al. (1997)
Yeast(*S.cer.* BJ-ECZ) hER(S)+β-gal(S)	17α-Ethinyl estradiol	57-63-6	pos.	120	0.00062				Le Guevel and Pakdel (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	17α-Ethinyl estradiol	57-63-6	pos.	147	0.0036				Le Guevel and Pakdel (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	S-Ethyl dipropylthiocarbamate	759-94-4	pos.	16.76					Petit et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	2-Ethylhexyl isodecyl phthalate	53272-22-3	neg.						Harris et al. (1997)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Ethylhexyl salicylate	118-60-5	pos.	<0.0000001					Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Ethylparaben	120-47-8	pos.	0.000005					Miller et al. (2001)
MCF-7 hER(E)+CP	4-Ethylphenol	123-07-9				neg.			Soto et al. (1995)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-Ethylphenol	123-07-9	neg.						Routledge and Sumpter (1997)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Ethyl vanillin	121-32-4	neg.						Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Eugenol	97-53-0	neg.						Miller et al. (2001)
BG-1 hER(E)+Luc(S)	Famphur	52-85-7	neg.						Xenobiotic Detection Systems (2001)
MCF-7(E3) hER(E)+CP	Fenarimol	60168-88-9			2	pos.	pos.		Vinggaard et al. (1999)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Fenarimol	60168-88-9	pos.	0.000003	12				Vinggaard et al. (1999)
CHO-K1 hERα(T)+Luc(T)†	Fenbuconazole	14369-43-6	neg.		0				Otsuka Pharmaceutical (2001)
HeLa hERα(T)+Luc(T)	Fenvalerate	51630-58-1	neg.	9.6					Sumida et al. (2001)
MCF-7 hER(E)+CP	Fenvalerate	51630-58-1				pos.			Go et al. (1999)
HEK-293 hERα(T)+Luc(T)	Flavone	525-82-6	neg.	0			pos.	0	Collins-Burow et al. (2000)
HEK-293 hERα(T)+Luc(T)	Flavone	525-82-6	neg.	0			pos.	35.1	Collins-Burow et al. (2000)

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HEK293 hERα(T)+Luc(T)+β-gal(T)	Flavone	525-82-6	pos.	2					Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	Flavone	525-82-6	pos.	2					Kuiper et al. (1998)
MCF-7 hER(E)+CP	Flavone	525-82-6				neg.			Miksicek (1993)
MCF-7(M) hER(E)+Luc(T)+β-gal(T)	Flavone	525-82-6	neg.	0			pos.		Collins-Burow et al. (2000)
MCF-7(M) hER(E)+CP	Flavone	525-82-6				neg.	pos.		Collins-Burow et al. (2000)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Fluazifop-butyl	69806-50-4	pos.	16.87					Petit et al. (1997)
MCF-7 hER(E)+CP(F)	Fluoranthene	206-44-0				neg.	neg.		Arcaro et al. (1999b)
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	Fluoranthene	206-44-0	neg.				neg.	96	Tran et al. (1996)
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	Fluoranthene	206-44-0					neg.		Tran et al. (1996)
BG-1 hER(E)+Luc(S)	Fluorene	86-73-7	pos.						Xenobiotic Detection Systems (2001)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Fluorene	86-73-7	neg.		0				Clemons et al. (1998)
MCF-7 hER(E)+CP(F)	Fluorene	86-73-7				neg.	neg.		Arcaro et al. (1999b)
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	Fluorene	86-73-7	neg.				neg.	101	Tran et al. (1996)
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	Fluorene	86-73-7					neg.		Tran et al. (1996)
CHO-K1 hERα(T)+Luc(T)†	Flutamide	13311-84-7	neg.		0				Otsuka Pharmaceutical (2001)
HEK293 hERα(T)+Luc(T)+β-gal(T)	Formononetin	485-72-3	pos.	6			neg.		Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	Formononetin	485-72-3	pos.	2			neg.		Kuiper et al. (1998)
HeLa hER(T)+CAT(T)	Formononetin	485-72-3	pos.	58.75	0.3				Miksicek (1994)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Formononetin	485-72-3	pos.	0.0056					Coldham et al. (1997)

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Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Formononetin	485-72-3	neg.				pos.		Morito et al. (2001b)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Formononetin	485-72-3	pos.		16		pos.		Morito et al. (2001b)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Gallic acid	149-91-7	neg.						Miller et al. (2001)
BG-1 hER(E)+Luc(S)	Genistein	446-72-0	pos.	0.016	0.062				Xenobiotic Detection Systems (2001)
BG-1 hER(E)+Luc(T)	Genistein	446-72-0	pos.	70					Rogers and Denison (2000)
CHO-K1 hERα(S)+Luc(S)†	Genistein	446-72-0	pos.		0.0273				Otsuka Pharmaceutical (2001)
CHO-K1 hERα(T)+Luc(T)†	Genistein	446-72-0	pos.		0.00423				Otsuka Pharmaceutical (2001)
HEK293 hERα(T)+Luc(T)+β-gal(T)	Genistein	446-72-0	pos.	198			neg.		Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	Genistein	446-72-0	pos.	182			neg.		Kuiper et al. (1998)
HeLa hER(T)+CAT(T)	Genistein	446-72-0	pos.	123.75	0.09				Miksicek (1994)
HeLa mER(T)+CAT(T)	Genistein	446-72-0		26		pos.			Makela et al. (1994)
HeLa mER(T)+CAT(T)*	Genistein	446-72-0	pos.	17x (100 nM)					Makela et al. (1994)
MCF-7 hER(E)+CP	Genistein	446-72-0				pos.			Dodge et al. (1996)
MCF-7 hER(E)+CP	Genistein	446-72-0				pos.	neg.		Makela et al. (1994)
MCF-7 hER(E)+CP	Genistein	446-72-0				neg.	pos.		Miodini et al. (1999)
MCF-7 hER(E)+CP	Genistein	446-72-0				pos.			Morito et al. (2001a)
MCF-7(M) hER(E)+Luc(T)+β-gal(T)	Genistein	446-72-0	pos.	83			pos.		Collins-Burow et al. (2000)
T47D hER(E)+CP	Genistein	446-72-0				pos.	neg.		Makela et al. (1994)
T47D hER(E)+Luc(S)	Genistein	446-72-0	pos.	0.06	0.1				Legler et al. (1999)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Genistein	446-72-0	pos.		76.4-1.40				De Boever et al. (2001)

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Yeast(*S.cer.*) hER(S)+β-gal(S)	Genistein	446-72-0	pos.	0.0001					Harris et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Genistein	446-72-0	pos.	0.0008	0.038				Routledge and Sumpter (1996)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Genistein	446-72-0	pos.	0.049					Coldham et al. (1997)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Genistein	446-72-0	pos.		3				Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Genistein	446-72-0	pos.		16		neg.		Morito et al. (2001b)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Genistein	446-72-0	pos.		0.1				Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Genistein	446-72-0	pos.		0.25		neg.		Morito et al. (2001b)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	5-OMe-Genistein		neg.				pos.		Morito et al. (2001b)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	5-OMe-Genistein		pos.		20		pos.		Morito et al. (2001b)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	7-OMe-Genistein		neg.				pos.		Morito et al. (2001b)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	7-OMe-Genistein		pos.				pos.		Morito et al. (2001b)
MCF-7 hER(E)+CP	Genistin	529-59-9				pos.			Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Genistin	529-59-9	neg.						Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Genistin	529-59-9	neg.				neg.		Morito et al. (2001b)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Genistin	529-59-9	pos.		10				Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Genistin	529-59-9	neg.				neg.		Morito et al. (2001b)
HeLa hER(T)+CAT(T)	Gibberellic acid	77-06-5	neg.	7.25					Miksicek (1994)
MCF-7 hER(E)+CP(F)	Glabridin	59870-68-7				pos.	neg.		Tamir et al. (2000)
T47D hER(E)+CP	Glabridin	59870-68-7				pos.	neg.		Tamir et al. (2000)

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MCF-7 hER(E)+CP	Glycitein	40957-83-3				neg.			Morito et al. (2001a)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Glycitein	40957-83-3	neg.						De Boever et al. (2001)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Glycitein	40957-83-3	pos.		30				Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Glycitein	40957-83-3	pos.		1				Morito et al. (2001a)
MCF-7 hER(E)+CP	Glycitin					neg.			Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Glycitin		neg.						Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Glycitin		neg.						Morito et al. (2001a)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Glyphosate	1071-83-6	pos.	15.84					Petit et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	HEPES	7365-45-9	neg.				neg.		Moffat et al. (2001)
BG-1 hER(E)+Luc(S)	Heptachlor	76-44-8	neg.						Xenobiotic Detection Systems (2001)
MCF-7 hER(E)+CP	Heptachlor	76-44-8				neg.			Soto et al. (1995)
MCF-7(E3) hER(E)+CP	Heptachlor	76-44-8				neg.			Vinggaard et al. (1999)
MCF-7 hER(E)+CAT(T)+β-gal(T)	2,2',3,4,4',5,5'-Heptachlorobiphenyl	35065-29-3	neg.				pos.	65	Bonefeld-Jørgensen et al. (2001)
MCF-7 hER(E)+CP	2,2',3,4,4',5,5'-Heptachlorobiphenyl	35065-29-3				neg.	pos.		Bonefeld-Jørgensen et al. (2001)
MDA-MB-231 hERα(T)+Luc(T)	2,2',3,4,4',5,5'-Heptachlorobiphenyl	35065-29-3					pos.		Bonefeld-Jørgensen et al. (2001)
HeLa hERαdef(S)+Luc(S)	2,2',3,3',4',5,5'-Heptachloro-4-biphenylol	158076-64-3	weak	9			pos.		Moore et al. (1997)
HeLa hERαdef(S)+Luc(S)	2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8	weak	3			pos.		Moore et al. (1997)
MCF-7 hER(E)+CP	2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	158076-69-8				neg.	pos.		Moore et al. (1997)
HeLa hERαdef(S)+Luc(S)	2,2',3,4',5,5',6-Heptachloro-4-biphenylol	158076-68-7	pos.	7			pos.		Moore et al. (1997)
MCF-7 hER(E)+CP	2,2',3,4',5,5',6-Heptachloro-4-biphenylol	158076-68-7				neg.	pos.		Moore et al. (1997)

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Yeast(*S.cer.*) hER(S)+β-gal(S)	4-*n* -Heptylphenol	1987-50-4	pos.	0.000015	13.3				Routledge and Sumpter (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-*tert* -Heptylphenol		pos.	0.0003	0.6				Routledge and Sumpter (1997)
BG-1 hER(E)+Luc(S)	Hexachlorobenzene	118-74-1	neg.						Xenobiotic Detection Systems (2001)
MCF-7 hER(E)+CP	Hexachlorobenzene	118-74-1				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP	2,2',3,3',5,5'-Hexachlorobiphenyl	35694-04-3				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP	2,2',3,3',6,6'-Hexachlorobiphenyl	38411-22-2				neg.			Soto et al. (1994)
MCF-7 hER(E)+CP	2,2',3,3',6,6'-Hexachlorobiphenyl	38411-22-2				pos.			Soto et al. (1995)
MCF-7 hER(E)+CAT(T)+β-gal(T)	2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2	neg.				pos.	36	Bonefeld-Jørgensen et al. (2001)
MCF-7 hER(E)+CP	2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2				neg.	pos.		Bonefeld-Jørgensen et al. (2001)
MDA-MB-231 hERα(T)+Luc(T)	2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2					pos.		Bonefeld-Jørgensen et al. (2001)
MCF-7 hER(E)+CAT(T)+β-gal(T)	2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	neg.				pos.	41	Bonefeld-Jørgensen et al. (2001)
MCF-7 hER(E)+CP	2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1				neg.	pos.		Bonefeld-Jørgensen et al. (2001)
MDA-MB-231 hERα(T)+Luc(T)	2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1					pos.		Bonefeld-Jørgensen et al. (2001)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	2,2',4,4',6,6'-Hexachlorobiphenyl	33979-03-2		0			pos.		Fielden et al. (1997)
MCF-7 hER(E)+CP	2,2',4,4',6,6'-Hexachlorobiphenyl	33979-03-2				pos.	pos.		Fielden et al. (1997)
HeLa hERαdef(S)+Luc(S)	2,2',3,3',4',5-Hexachloro-4-biphenylol	158076-62-1	neg.	3			neg.		Moore et al. (1997)
MCF-7 hER(E)+CP	2,2',3,3',4',5-Hexachloro-4-biphenylol	158076-62-1				neg.	pos.		Moore et al. (1997)
HeLa hERαdef(S)+Luc(S)	2,2',3,4',5,5'-Hexachloro-4-biphenylol	145413-90-7	neg.	0			pos.		Moore et al. (1997)
MCF-7 hER(E)+Luc(S)	2',3,3',4',5,5'-Hexachloro-4-biphenylol	158076-63-2			3.8		pos.		Kramer et al. (1997)
MCF-7 hER(E)+CP	β-Hexachlorocyclohexane	319-85-7			3	pos.			Payne et al. (2001)
CHO-K1 hERα(T)+Luc(T)†	β-Hexachlorocyclohexane	319-85-7	neg.		0				Otsuka Pharmaceutical (2001)

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HEK293 hERα(S)+Luc(T)	1,2,4,6,7,8-Hexahydro-4,6,6,7,8-hexamethylcyclopenta-*y* -2-benzopyrene		neg.						Seinen et al. (1999)
HEK293 hERα(T)+Luc(T)	1,2,4,6,7,8-Hexahydro-4,6,6,7,8-hexamethylcyclopenta-*y* -2-benzopyrene		pos.						Seinen et al. (1999)
HEK293 hERβ(S)+Luc(T)	1,2,4,6,7,8-Hexahydro-4,6,6,7,8-hexamethylcyclopenta-*y* -2-benzopyrene		neg.						Seinen et al. (1999)
HEK293 hERβ(T)+Luc(T)	1,2,4,6,7,8-Hexahydro-4,6,6,7,8-hexamethylcyclopenta-*y* -2-benzopyrene		neg.						Seinen et al. (1999)
MCF-7 hER(E)+CP	Hexazinone	51235-04-2				neg.			Soto et al. (1995)
HeLa hER(T)+CAT(T)	Hexestrol	84-16-2	pos.		0.0002				Miksicek (1993)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Hexestrol	84-16-2	pos.	30.6					Coldham et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Hexyl decyl phthalate		neg.						Harris et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Hexyl 2-ethylhexyl phthalate		neg.						Harris et al. (1997)
MCF-7 hER(E)+CP	4-Hexyloxyphenol	18979-55-0				neg.			Soto et al. (1995)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-*tert* -Hexylphenol	2446-69-7	pos.	0.0002	1				Routledge and Sumpter (1997)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Hexyl salicylate	6259-76-3	neg.						Miller et al. (2001)
MCF-7(Bos) hER(E)+CP	Homosalate	118-56-9		36.81	1.56	pos.			Schlumpf et al. (2001)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	3-Hydroxybenzo[β]naphtho(2,3-*d*)thiophene		weak						Fertuck et al. (2001b)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	3-Hydroxybenzo[β]naphtho(2,3-*d*)thiophene		weak						Fertuck et al. (2001b)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	2-Hydroxybenzo[*c*]phenanthrene	22717-94-8	weak						Fertuck et al. (2001b)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	2-Hydroxybenzo[*c*]phenanthrene	22717-94-8	weak						Fertuck et al. (2001b)

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MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	3-Hydroxy-benzo[β]phenanthro(2,3-*d*)thiophene		pos.	2.2	0.16				Fertuck et al. (2001b)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	3-Hydroxy-benzo[β]phenanthro(2,3-*d*)thiophene		pos.	3.25	0.04				Fertuck et al. (2001b)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	1-Hydroxybenzo[*a*]pyrene	13345-23-8		0.004	3.2				Fertuck et al. (2001a)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	1-Hydroxybenzo[*a*]pyrene	13345-23-8		0.004	3.2				Fertuck et al. (2001a)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	3-Hydroxybenzo[*a*]pyrene	13345-21-6	pos.	1.6					Charles et al. (2000b)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	3-Hydroxybenzo[*a*]pyrene	13345-21-6	[a]wi						Fertuck et al. (2001a)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	3-Hydroxybenzo[*a*]pyrene	13345-21-6		0.062	0.21				Fertuck et al. (2001a)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	(+-)-*trans* -7,8-Hydroxybenzo[*a*]pyrene	61443-57-0	neg.	0					Charles et al. (2000b)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	7-Hydroxybenzo[*a*]pyrene	37994-82-4	wi						Fertuck et al. (2001a)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	7-Hydroxybenzo[*a*]pyrene	37994-82-4		0.03	0.43				Fertuck et al. (2001a)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	*trans* -9,10-Hydroxybenzo[*a*]pyrene		pos.	0.2					Charles et al. (2000b)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	9-Hydroxybenzo[*a*]pyrene	17573-21-6	pos.	1.12					Charles et al. (2000b)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	9-Hydroxybenzo[*a*]pyrene	17573-21-6		0.011	1.2				Fertuck et al. (2001a)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	9-Hydroxybenzo[*a*]pyrene	17573-21-6		0.03	0.43				Fertuck et al. (2001a)
MCF-7 hER(E)+CP	3-Hydroxybisphenol A					pos.			Nakagawa and Suzuki (2001)

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MCF-7 hERα(E)+CP	1-Hydroxychlordene	2597-11-7				pos.			Soto et al. (1995)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	2-Hydroxychrysene	65945-06-4	weak						Fertuck et al. (2001b)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	2-Hydroxychrysene	65945-06-4	pos.	2.1	0.063				Fertuck et al. (2001b)
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	6-Hydroxychrysene	37515-51-8	neg.				pos.	74	Tran et al. (1996)
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	6-Hydroxychrysene	37515-51-8					pos.	71	Tran et al. (1996)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	7-Hydroxycoumarin	93-35-6	neg.						Miller et al. (2001)
HeLa mER(T)+CAT(T)	2-Hydroxyestradiol	362-05-0	pos.	29.42					Garner et al. (1999)
T47D hER(E)+Luc(T)	2-Hydroxyestradiol	362-05-0	pos.	0.015					Hoogenboom et al. (2001)
Yeast(*S.cer* .) hERα(S)+β-gal(S)	2-Hydroxyestradiol	362-05-0	pos.	0.003	0.0195				Elsby et al. (2001)
HeLa mER(T)+CAT(T)	4-Hydroxyestradiol	5976-61-4	pos.	38.78					Garner et al. (1999)
T47D hER(E)+Luc(T)	4-Hydroxyestradiol	5976-61-4	pos.	45					Hoogenboom et al. (2001)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	2-Hydroxyestriol	1232-80-0	pos.	0.0048	2				Charles et al. (2000a)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	16α-Hydroxyestriol		pos.	9.6	0.001				Charles et al. (2000a)
T47D hER(E)+Luc(T)	2-Hydroxyestrone	362-06-1	pos.	0.065					Hoogenboom et al. (2001)
T47D hER(E)+Luc(T)	4-Hydroxyestrone	3131-23-5	pos.	0.54					Hoogenboom et al. (2001)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	11β-Hydroxyestrone	6803-21-0	pos.	0.096	0.1				Charles et al. (2000a)
MCF-7 hER(E)+CP	16-Hydroxyestrone	18186-49-7				pos.			Soto et al. (1995)
Yeast(*S.cer.*) hER(S)+β-gal(S)	N-β-Hydroxyethyl oleyl imidazoline		neg.						Routledge and Sumpter (1996)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	2-Hydroxy-5-methylchrysene		weak						Fertuck et al. (2001b)

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MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	2-Hydroxy-5-methylchrysene		pos.	0.09	0.32				Fertuck et al. (2001b)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	8-Hydroxy-5-methylchrysene		weak						Fertuck et al. (2001b)
MCF-7 mERβdef(T)+Luc(T)+β-gal(T)	8-Hydroxy-5-methylchrysene		weak						Fertuck et al. (2001b)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	*N* -(4-Hydroxyphenyl)stearamide	103-99-1	neg.						Miller et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-Hydroxyprogesterone	650-66-8	neg.	0					Routledge and Sumpter (1997)
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	1-Hydroxypyrene	5315-79-7	neg.				neg.	104	Tran et al. (1996)
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	1-Hydroxypyrene	5315-79-7					neg.		Tran et al. (1996)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	8-Hydroxyquinoline	148-24-3	neg.						Miller et al. (2001)
CHO-K1 hERα(T)+Luc(T)†	4-Hydroxytamoxifen	68047-06-3	neg.		0		pos.		Otsuka Pharmaceutical (2001)
COS-1 mERα(T)+Lucb(T)	4-Hydroxytamoxifen	68047-06-3	pos.				pos.		Tremblay et al. (1998)
COS-1 mERα(T)+Luctk(T)	4-Hydroxytamoxifen	68047-06-3	neg.				pos.		Tremblay et al. (1998)
COS-1 mERβ(T)+Lucb(T)	4-Hydroxytamoxifen	68047-06-3	neg.				pos.		Tremblay et al. (1998)
COS-1 mERβ(T)+Luctk(T)	4-Hydroxytamoxifen	68047-06-3	neg.				pos.		Tremblay et al. (1998)
HEK-293 hERα(T)+Luc(T)	4-Hydroxytamoxifen	68047-06-3							Collins-Burow et al. (2000)
HEK-293 hERα(T)+Luc(T)	4-Hydroxytamoxifen	68047-06-3							Collins-Burow et al. (2000)
HeLa hER(T)+CAT(T)	4-Hydroxytamoxifen	68047-06-3					pos.		Miksicek (1994)
HeLa mER(T)+CAT(T)*	4-Hydroxytamoxifen	68047-06-3	pos.	36x (10 nM)					Shelby et al. (1996)
HepG2 hERα(T)+Luc(T)+β-gal(T)	4-Hydroxytamoxifen	68047-06-3	pos.				pos.		Gould et al. (1998)
MCF-7(M) hER(E)+Luc(T)+β-gal(T)	4-Hydroxytamoxifen	68047-06-3	neg.	0			pos.		Collins-Burow et al. (2000)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
MCF-7 hER(E)+Luc(T)+β-gal(T)	4-Hydroxytamoxifen	68047-06-3					pos.		Jobling et al. (1995)
MCF-7 hER(E)+Luc(T)+β-gal(T)	4-Hydroxytamoxifen	68047-06-3					pos.		Klotz et al. (1996)
MCF-7(M) hER(E)+CP	4-Hydroxytamoxifen	68047-06-3					pos.		Collins-Burow et al. (2000)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	4-Hydroxytamoxifen	68047-06-3	neg.				pos.		Beresford et al. (2000)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-Hydroxytamoxifen	68047-06-3					pos.		Moffat et al. (2001)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	4-Hydroxytamoxifen	68047-06-3	pos.	0.0073			pos.		Coldham et al. (1997)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	4-Hydroxytamoxifen	68047-06-3	neg.				pos.		Morito et al. (2001b)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	4-Hydroxytamoxifen	68047-06-3	neg.				pos.		Morito et al. (2001b)
MCF-7 hER(E)+CP	6-Hydroxytetralin	1125-78-6				neg.			Soto et al. (1995)
Yeast(*S.cer* .) hERα(S)+β-gal(S)	6-Hydroxytetralin	1125-78-6	pos.	0.0000007	70				Elsby et al. (2001)
COS-1 mERα(T)+Lucb(T)	Hydroxytoremifene	110503-62-3	pos.				pos.		Tremblay et al. (1998)
COS-1 mERα(T)+Luctk(T)	Hydroxytoremifene	110503-62-3	neg.				pos.		Tremblay et al. (1998)
COS-1 mERβ(T)+Lucb(T)	Hydroxytoremifene	110503-62-3	neg.				pos.		Tremblay et al. (1998)
COS-1 mERβ(T)+Luctk(T)	Hydroxytoremifene	110503-62-3	neg.				pos.		Tremblay et al. (1998)
HeLa hER(S)+Luc(S)	ICI 164,384	98007-99-9					pos.		Balaguer et al. (1996)
HeLa hER(T)+CAT(T)	ICI 164,384	98007-99-9	neg.	6.7			pos.		Miksicek (1993)
HeLa hER(T)+CAT(T)	ICI 164,384	98007-99-9					pos.		Miksicek (1994)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	ICI 164,384	98007-99-9							Clemons et al. (1998)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	ICI 164,384	98007-99-9					pos.		Fielden et al. (1997)
MCF-7 hER(E)+CP	ICI 164,384	98007-99-9				neg.			Miksicek (1993)

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MCF-7(E3) hER(E)+CP	ICI 164,384	98007-99-9					pos.		Wiese et al. (1997)
MCF-7(MELN41) hER(E)+Luc(S)	ICI 164,384	98007-99-9					pos.		Lascombe et al. (2000)
Yeast(*S.cer* .939) hER(S)+β-gal(S)	ICI 164,384	98007-99-9	pos.	0.003			pos.	18.4	Chen et al. (1997)
Yeast(*S.cer.* BJ2407) hER(S)+β-gal(S)	ICI 164,384	98007-99-9	neg.						Arnold et al. (1996)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	ICI 164,384	98007-99-9	pos.	0.00002	14.5				Gaido et al. (1997)
Yeast(*S.cer.* CYT10-5d) hER(S)+β-gal(S)	ICI 164,384	98007-99-9	neg.	0			pos.	65	Chen et al. (1997)
COS-1 mERα(T)+Lucb(T)	ICI 182,780	129453-61-8	neg.				pos.		Tremblay et al. (1998)
COS-1 mERα(T)+Luctk(T)	ICI 182,780	129453-61-8	neg.				pos.		Tremblay et al. (1998)
COS-1 mERβ(T)+Lucb(T)	ICI 182,780	129453-61-8	neg.				pos.		Tremblay et al. (1998)
COS-1 mERβ(T)+Luctk(T)	ICI 182,780	129453-61-8	neg.				pos.		Tremblay et al. (1998)
ELT-3 hER(T)+Luc(T)+β-gal(T)	ICI 182,780	129453-61-8					pos.		Hodges et al. (2000)
HEK293 hERα(T)+Luc(T)+β-gal(T)	ICI 182,780	129453-61-8	neg.	1					Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	ICI 182,780	129453-61-8	pos.	2			neg.		Kuiper et al. (1998)
HeLa mER(T)+CAT(T)	ICI 182,780	129453-61-8					pos.		Garner et al. (1999)
HepG2 hERα(T)+Luc(T)+β-gal(T)	ICI 182,780	129453-61-8	neg.				pos.		Gould et al. (1998)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	ICI 182,780	129453-61-8	neg.	0			pos.		Charles et al. (2000a)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	ICI 182,780	129453-61-8	neg.				pos.		Charles et al. (2000b)
MCF-7 hER(E)+CAT(T)+β-gal(T)	ICI 182,780	129453-61-8	neg.				pos.	90	Bonefeld-Jørgensen et al. (2001)
MCF-7 hER(E)+CP	ICI 182,780	129453-61-8							Bonefeld-Jørgensen et al. (2001)

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MCF-7 hER(E)+Luc(T)+β-gal(T)	ICI 182,780	129453-61-8					pos.		Jobling et al. (1995)
MCF-7(Bos) hER(E)+CP	ICI 182,780	129453-61-8					pos.		Schlumpf et al. (2001)
MCF-7(E3) hER(E)+CP	ICI 182,780	129453-61-8					pos.		Vinggaard et al. (1999)
MCF-7(McGrath) hER(E)+CP	ICI 182,780	129453-61-8				neg.	pos.		Nakagawa and Suzuki (2001)
MDA-MB-231 hERα(T)+Luc(T)	ICI 182,780	129453-61-8					pos.		Bonefeld-Jørgensen et al. (2001)
T47D hER(E)+Luc(S)	ICI 182,780	129453-61-8					pos.		Legler et al. (1999)
T47D hER(E)+Luc(S)	ICI 182,780	129453-61-8					pos.	0.00001	Meerts et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	ICI 182,780	129453-61-8	pos.	0.00003			neg.		Beresford et al. (2000)
Yeast(*S.cer.* YRG-2) hERα(S)+β-gal(S)	ICI 182,780	129453-61-8	pos.	5x (1 µM)*			neg.		Lascombe et al. (2000)
ZR-75 hER(E)+CP	ICI 182,780	129453-61-8							Nakagawa and Suzuki (2001)
MCF-7(E3) hER(E)+CP	Imazalil	35554-44-0				neg.			Vinggaard et al. (1999)
MCF-7 hER(E)+CP	Indanestrol	71855-45-3				pos.			Soto et al. (1995)
MCF-7 hER(E)+CP	Indanestrol-A					pos.			Soto et al. (1995)
MCF-7 hER(E)+CP	Indanestrol-B					pos.			Soto et al. (1995)
BG-1 hER(E)+Luc(S)	Indeno(1,2,3,*cd*)pyrene	193-39-5	neg.						Xenobiotic Detection Systems (2001)
MCF-7 hER(E)+CP(F)	Indeno(1,2,3,*cd*)pyrene	193-39-5				neg.	pos.		Arcaro et al. (1999b)
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	Indeno(1,2,3,*cd*)pyrene	193-39-5	neg.				neg.	101	Tran et al. (1996)
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	Indeno(1,2,3,*cd*)pyrene	193-39-5					neg.		Tran et al. (1996)
MCF-7(E3) hER(E)+CP	2-Iodoestratrien-17β-ol	107900-35-6			1.7	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	3-Iodoestratrien-17β-ol	38605-46-8			0.174	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	4-Iodoestratrien-17β-ol	107900-36-7			1.67	pos.			Wiese et al. (1997)

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HEK293 hERα(T)+Luc(T)+β-gal(T)	Ipriflavone	35212-22-7	pos.	11			neg.		Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	Ipriflavone	35212-22-7	pos.	3			neg.		Kuiper et al. (1998)
MCF-7(E3) hER(E)+CP	Iprodion	36734-19-7				neg.			Vinggaard et al. (1999)
MCF-7 hER(E)+CP	Irganox 1640					neg.			Soto et al. (1995)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Irisolidone		neg.				pos.		Morito et al. (2001b)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Irisolidone		pos.		25		pos.		Morito et al. (2001b)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Irisolidone-7-*O* -beta-*D* -glucoside		neg.				neg.		Morito et al. (2001b)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Irisolidone-7-*O* -beta-*D* -glucoside		neg.				neg.		Morito et al. (2001b)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Isodecyl tridecyl phthalate	61886-60-0	neg.						Harris et al. (1997)
BG-1 hER(E)+Luc(S)	Isodrin	465-73-6	pos.						Xenobiotic Detection Systems (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Isoeugenol	97-54-1	neg.						Miller et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Isohexylbenzyl phthalate	1242-92-8	neg.						Harris et al. (1997)
HeLa hER(T)+CAT(T)	Isoliquiritigenin	961-29-5	pos.	79					Miksicek (1993)
MCF-7 hER(E)+CP	4-Isopentylphenol	1805-61-4				pos.			Soto et al. (1995)
MCF-7 hER(E)+CP	Isorhapontigenin	32507-66-7				pos.			Mellanen et al. (1996)
MCF-7 hER(E)+CP	Isorhapontin	32727-29-0				pos.			Mellanen et al. (1996)
HEK-293 hERα(T)+Luc(T)	Kaempferide	491-54-3	neg.	0			pos.	8.9	Collins-Burow et al. (2000)
HEK-293 hERα(T)+Luc(T)	Kaempferide	491-54-3	neg.	0			pos.	8.1	Collins-Burow et al. (2000)
MCF-7(M) hER(E)+Luc(T)+β-gal(T)	Kaempferide	491-54-3	pos.	35			pos.		Collins-Burow et al. (2000)
MCF-7(M) hER(E)+CP	Kaempferide	491-54-3				neg.	pos.		Collins-Burow et al. (2000)

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HEK293 hERα(T)+Luc(T)+β-gal(T)	Kaempferol	520-18-3	pos.	35			neg.		Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	Kaempferol	520-18-3	pos.	53			neg.		Kuiper et al. (1998)
MCF-7(M) hER(E)+Luc(T)+β-gal(T)	Kaempferol	520-18-3					neg.		Collins-Burow et al. (2000)
BG-1 hER(E)+Luc(T)	Kepone	143-50-0	pos.	30					Rogers and Denison (2000)
CHO-K1 hERα(T)+Luc(T)†	Kepone	143-50-0	neg.		0				Otsuka Pharmaceutical (2001)
ELT-3 hER(T)+Luc(T)+β-gal(T)	Kepone	143-50-0	pos.	12.62					Hodges et al. (2000)
HEK293 hERα(T)+Luc(T)+β-gal(T)	Kepone	143-50-0	pos.	27			neg.		Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	Kepone	143-50-0	neg.	1			neg.		Kuiper et al. (1998)
HeLa mER(T)+CAT(T)*	Kepone	143-50-0	pos.	6 (1 µM)					Shelby et al. (1996)
MCF-7 hER(E)+CP	Kepone	143-50-0				pos.			Soto et al. (1994)
MCF-7 hER(E)+CP	Kepone	143-50-0				pos.			Soto et al. (1995)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Kepone	143-50-0	pos.	28.36					Petit et al. (1997)
MCF-7(E3) hER(E)+CP	6-Ketoestradiol	571-92-6			0.00338	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	7-Ketoestratriene-3,17β-diol	3398-12-7			0.016	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	11-Ketoestratriene-3,17β-diol	571-65-3			8.97	pos.			Wiese et al. (1997)
CHO-K1 hERα(T)+Luc(T)†	11-Ketotestosterone	564-35-2	neg.		0				Otsuka Pharmaceutical (2001)
CHO-K1 hERα(S)+Luc(S)†	Levonorgestrel	797-63-7	neg.		0				Otsuka Pharmaceutical (2001)
CHO-K1 hERα(T)+Luc(T)†	Levonorgestrel	797-63-7	pos.		0.33				Otsuka Pharmaceutical (2001)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Levonorgestrel	797-63-7	pos.	0.0004					Coldham et al. (1997)
BG-1 hER(E)+Luc(S)	Lindane	58-89-9	pos.						Xenobiotic Detection Systems (2001)

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CHO-K1 hERα(T)+Luc(T)†	Lindane	58-89-9	neg.		0				Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Lindane	58-89-9				neg.			Soto et al. (1995)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Lindane	58-89-9	pos.	47.75					Petit et al. (1997)
CHO-K1 hERα(T)+Luc(T)†	Linuron	330-55-2	neg.		0				Otsuka Pharmaceutical (2001)
MCF-7(E3) hER(E)+CP	Linuron	330-55-2				neg.			Vinggaard et al. (1999)
MCF-7(M) hER(E)+Luc(T)+β-gal(T)	Luteolin	491-70-3	pos.	25			pos.		Collins-Burow et al. (2000)
MCF-7(M) hER(E)+CP	Luteolin	491-70-3					pos.		Collins-Burow et al. (2000)
MCF-7 hER(E)+CP	Malathion	121-75-5				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP	Maneb	12427-38-2				neg.			Soto et al. (1995)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Melengesterol acetate	2919-66-6	pos.	4.4	0.11				Le Guevel and Pakdel (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Menthyl salicylate	89-46-3	pos.	<0.000005					Miller et al. (2001)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Mestranol	72-33-3	pos.	7.3					Coldham et al. (1997)
Ishikawa hER(T)+Luc(T)+β-gal(T)	Methomyl	16752-77-5	neg.	0.06			pos.	36%	Klotz et al. (1997)
MCF-7 hER(E)+Luc(T)+β-gal(T)	Methomyl	16752-77-5		0.06				28%	Klotz et al. (1997)
MCF-7 hER(E)+CP	Methoprene	40596-69-8				neg.			Soto et al. (1995)
Yeast(*S.cer* .) hERα(S)+β-gal(S)	Methoxybisphenol A		neg.				pos.		Elsby et al. (2001)
BG-1 hER(E)+Luc(S)	Methoxychlor	72-43-5	pos.						Xenobiotic Detection Systems (2001)
CHO-K1 hERα(T)+Luc(T)†	Methoxychlor	72-43-5	neg.		0				Otsuka Pharmaceutical (2001)
ELT-3 hER(T)+Luc(T)+β-gal(T)	Methoxychlor	72-43-5	pos.	10.55					Hodges et al. (2000)

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HEK293 hERα(T)+Luc(T)+β-gal(T)	Methoxychlor	72-43-5	pos.	9					Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	Methoxychlor	72-43-5	pos.	2					Kuiper et al. (1998)
HeLa hERα(T)+Luc(T)	Methoxychlor	72-43-5	pos.	81					Sumida et al. (2001)
HeLa mER(T)+CAT(T)*	Methoxychlor	72-43-5	pos.	6 (1 µM)					Shelby et al. (1996)
HepG2 hERα(T)+Luc(T)+β-gal(T)	Methoxychlor	72-43-5	pos.	25			neg.	85	Gaido et al. (2000)
HepG2 hERβ(T)+Luc(T)+β-gal(T)	Methoxychlor	72-43-5	pos.	10			neg.	75µM	Gaido et al. (2000)
MCF-7 hER(E)+CP	Methoxychlor	72-43-5				pos.			Dodge et al. (1996)
MCF-7 hER(E)+CP	Methoxychlor	72-43-5				neg.			Soto et al. (1994)
MCF-7 hER(E)+CP	Methoxychlor	72-43-5				pos.			Soto et al. (1995)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Methoxychlor	72-43-5	pos.	0.0008	12				Charles et al. (2000a)
T47D hER(E)+Luc(S)	Methoxychlor	72-43-5	pos.	0.001	5.7				Legler et al. (1999)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Methoxychlor	72-43-5	pos.	0.00001					Beresford et al. (2000)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Methoxychlor	72-43-5	pos.		0.00066-10.3				De Boever et al. (2001)
Yeast(*S.cer* .) hERα(S)+β-gal(S)	Methoxychlor	72-43-5	pos.	0.00001	4.45		neg.		Elsby et al. (2001)
Yeast(*S.cer* .) hERα(S)+β-gal(S)	Methoxychlor	72-43-5	pos.	0.00001	4.38				Elsby et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Methoxychlor	72-43-5	neg.						Yoshihara et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)+S9*	Methoxychlor	72-43-5	pos.						Yoshihara et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Methoxychlor	72-43-5	pos.	0.000032	4.8				Odum et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Methoxychlor	72-43-5	pos.	0.0033					Coldham et al. (1997)

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Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Methoxychlor	72-43-5	pos.	0.0000001	1200				Gaido et al. (1997)
HeLa hERα(T)+Luc(T)+S9*	Methoxychlor	72-43-5	pos.	137					Sumida et al. (2001)
BG-1 hER(E)+Luc(T)	*o,p'* -Methoxychlor	30667-99-3	pos.	18					Rogers and Denison (2000)
T47D hER(E)+Luc(T)	2-Methoxyestrone	362-08-3	pos.	0.15					Hoogenboom et al. (2001)
T47D hER(E)+Luc(T)	4-Methoxyestrone		pos.	0.14					Hoogenboom et al. (2001)
MCF-7(Bos) hER(E)+CP	3-(4-Methylbenzylidene)camphor	36861-47-9		79.54	3.02	pos.			Schlumpf et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	4,4'-Methylenebis(2,6-di-*tert* -butylphenol)	118-82-1	neg.						Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	2,2'-Methylenebis(4-methyl-6-*tert* -butylphenol)	119-47-1	neg.						Miller et al. (2001)
BG-1 hER(E)+Luc(S)	2-Methylnaphthalene	91-57-6	neg.						Xenobiotic Detection Systems (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-Methyl-2-nonylphenol		pos.	0.00003	6				Routledge and Sumpter (1997)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Methylparaben	99-76-3	pos.	0.0000003					Miller et al. (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Methyl parathion	298-00-0	pos.	19.1					Petit et al. (1997)
CHO-K1 hERα(T)+Luc(T)†	1-Methyl-1-phenylindan	79034-12-1					neg.		Otsuka Pharmaceutical (2001)
CHO-K1 hERα(T)+Luc(T)†+S9*	1-Methyl-1-phenylindan	79034-12-1					pos.		Otsuka Pharmaceutical (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Methyl salicylate	119-36-8	neg.						Miller et al. (2001)
CHO-K1 hERα(S)+Luc(S)†	Methyltestosterone	58-18-4	pos.		0.00573				Otsuka Pharmaceutical (2001)
CHO-K1 hERα(T)+Luc(T)†	Methyltestosterone	58-18-4	pos.		0.0158				Otsuka Pharmaceutical (2001)
T47D hER(E)+Luc(S)	Methytrienolone	965-93-5		-	0				Legler et al. (1999)
MCF-7 hER(E)+CP	Metiram	9006-42-2				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP	Metolachlor	51218-45-2				neg.			Soto et al. (1995)

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CHO-K1 hERα(T)+Luc(T)†	Mifepristone	84371-65-3	neg.		0				Otsuka Pharmaceutical (2001)
BG-1 hER(E)+Luc(S)	Mirex	2385-85-5	neg.						Xenobiotic Detection Systems (2001)
CHO-K1 hERα(T)+Luc(T)†	Mirex	2385-85-5	neg.		0				Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Mirex	2385-85-5				neg.			Soto et al. (1995)
T47D hER(E)+Luc(S)	3-Monobromobisphenol A		pos.		0.00002				Meerts et al. (2001)
HepG2 hERα(T)+Luc(T)+β-gal(T)	Monohydroxy-DDE		pos.	55	0.67		neg.	100	Gaido et al. (2000)
HepG2 hERβ(T)+Luc(T)+β-gal(T)	Monohydroxy-DDE		pos.	20			pos.	50µM	Gaido et al. (2000)
HepG2 hERα(T)+Luc(T)+β-gal(T)	Monohydroxymethoxychlor	28463-03-8	pos.	80	0.198		neg.	90	Gaido et al. (2000)
HepG2 hERβ(T)+Luc(T)+β-gal(T)	Monohydroxymethoxychlor	28463-03-8	pos.	15			pos.	50µM	Gaido et al. (2000)
MCF-7 hER(E)+CP	Moxestrol	34816-55-2				pos.			Soto et al. (1995)
CHO-K1 hERα(T)+Luc(T)†	Nafoxidine	1845-11-0	neg.		0				Otsuka Pharmaceutical (2001)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Nafoxidine	1845-11-0	neg.	0					Coldham et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Nafoxidine	1845-11-0	pos.	0.00003	7.72				Gaido et al. (1997)
BG-1 hER(E)+Luc(S)	Naphthalene	91-20-3	neg.						Xenobiotic Detection Systems (2001)
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	Naphthalene	91-20-3	neg.				neg.	100	Tran et al. (1996)
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	Naphthalene	91-20-3					neg.		Tran et al. (1996)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	α-Naphthoflavone	604-59-1	pos.	0.03			selective		Charles et al. (2000b)
MCF-7 hER(E)+CP	1-Naphthol	90-15-3				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP	2-Naphthol	135-19-3				neg.			Soto et al. (1995)

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BG-1 hER(E)+Luc(S)	1,2-Naphthoquinone	524-42-5	neg.						Xenobiotic Detection Systems (2001)
HEK293 hERα(T)+Luc(T)+β-gal(T)	Naringenin	480-41-1	pos.	36			neg.		Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	Naringenin	480-41-1	pos.	45			neg.		Kuiper et al. (1998)
HeLa hER(S)+Luc(S)	Naringenin	480-41-1	pos.		1				Balaguer et al. (1996)
HeLa hER(T)+CAT(T)	Naringenin	480-41-1	pos.	26.5					Miksicek (1993)
MCF-7(M) hER(E)+Luc(T)+β-gal(T)	Naringenin	480-41-1	pos.	55			pos.		Collins-Burow et al. (2000)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Niacinamide	98-92-0	neg.				neg.		Moffat et al. (2001)
MCF-7(E3) hER(E)+CP	2-Nitroestratriene-3,17β-diol	6298-51-7			33.5	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	4-Nitroestratriene-3,17β-diol	6936-94-3			0.9	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	1-Nitroestratrien-17β-ol	194068-94-3			60.4	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	2-Nitroestratrien-17β-ol	101772-26-3			29.3	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	3-Nitroestratrien-17β-ol	197068-95-4			2.16	pos.			Wiese et al. (1997)
MCF-7(E3) hER(E)+CP	4-Nitroestratrien-17β-ol	101772-25-2			34.2	pos.			Wiese et al. (1997)
ZR-75 hER(E)+CP	4-Nitrotoluene	99-99-0				neg.			Jobling et al. (1995)
MCF-7 hER(E)+Luc(T)+β-gal(T)	*cis* -Nonachlor	5103-73-1		0.09					Klotz et al. (1996)
Yeast(*S.cer.* BJ2407) hER(S)+β-gal(S)	*cis* -Nonachlor	5103-73-1		20x (10 µM)*			neg.		Klotz et al. (1996)
MCF-7 hER(E)+Luc(T)+β-gal(T)	*trans* -Nonachlor	39765-80-5		0.3					Klotz et al. (1996)
Yeast(*S.cer.* BJ2407) hER(S)+β-gal(S)	*trans* -Nonachlor	39765-80-5		12x (10 µM)*			neg.		Klotz et al. (1996)
MCF-7(MELN41) hER(E)+Luc(S)	Nonylphenol	84852-15-3	pos.						Lascombe et al. (2000)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Nonylphenol	84852-15-3	pos.	0.000004	5				Vinggaard et al. (2000)

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Yeast(*S.cer.*) hER(S)+β-gal(S)	Nonylphenol	84852-15-3	pos.	0.00013	1.2				Odum et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Nonylphenol	84852-15-3	pos.	0.00019	0.8				Odum et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Nonylphenol	84852-15-3	pos.	0.005					Coldham et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Nonylphenol	84852-15-3	pos.	0.0002	1.1				Gaido et al. (1997)
Yeast(*S.cer.* YRG-2) hERα(S)+β-gal(S)	Nonylphenol	84852-15-3	pos.	3.2x (10 µM)*					Lascombe et al. (2000)
BG-1 hER(E)+Luc(T)	*n*- Nonylphenol	25154-52-3	pos.	76					Rogers and Denison (2000)
HEK293 hERα(T)+Luc(T)+β-gal(T)	*n*- Nonylphenol	25154-52-3	pos.	62					Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	*n*- Nonylphenol	25154-52-3	pos.	34					Kuiper et al. (1998)
MCF-7 hER(E)+CP	*n*- Nonylphenol	25154-52-3				pos.			Morito et al. (2001a)
MCF-7 hER(E)+CP	*n*- Nonylphenol	25154-52-3				pos.			Soto et al. (1995)
Yeast(*S.cer.*) hER(S)+β-gal(S)	*n*- Nonylphenol	25154-52-3	pos.	0.000008	0.8		neg.		Moffat et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	*o* -Nonylphenol	136-83-4	neg.						Routledge and Sumpter (1997)
CHO-K1 hERα(S)+Luc(S)†	*p* -Nonylphenol	104-40-5	pos.		0.0356				Otsuka Pharmaceutical (2001)
CHO-K1 hERα(T)+Luc(T)†	*p* -Nonylphenol	104-40-5	pos.		0.0845				Otsuka Pharmaceutical (2001)
HeLa mER(T)+CAT(T)*	*p* -Nonylphenol	104-40-5	pos.	89 (5 µM)					Shelby et al. (1996)
MCF-7 hER(E)+CP	*p* -Nonylphenol	104-40-5				pos.	pos.		Jones et al. (1998)
MCF-7 hER(E)+CP	*p* -Nonylphenol	104-40-5				pos.			Korner et al. (1998)
MCF-7 hER(E)+CP	*p* -Nonylphenol	104-40-5				pos.			Morito et al. (2001a)
MCF-7 hER(E)+CP	*p* -Nonylphenol	104-40-5				pos.			Soto et al. (1995)
T47D hER(E)+Luc(S)	*p* -Nonylphenol	104-40-5	pos.	0.023	0.26				Legler et al. (1999)

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Yeast(*S.cer.*) hERα(S)+β-gal(S)	*p* -Nonylphenol	104-40-5	pos.	0.00025					Beresford et al. (2000)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	*p* -Nonylphenol	104-40-5	pos.	0.00025					Beresford et al. (2000)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	*p* -Nonylphenol	104-40-5	pos.	0.00025					Beresford et al. (2000)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	*p* -Nonylphenol	104-40-5	pos.	0.00025					Beresford et al. (2000)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	*p* -Nonylphenol	104-40-5	pos.						Yoshihara et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)+S9*	*p* -Nonylphenol	104-40-5	pos.						Yoshihara et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	*p* -Nonylphenol	104-40-5	pos.	0.000046					Harris et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	*p* -Nonylphenol	104-40-5	neg.						Odum et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	*p* -Nonylphenol	104-40-5	pos.	0.002	0.02				Routledge and Sumpter (1996)
Yeast(*S.cer.*) hER(S)+β-gal(S)	*p* -Nonylphenol	104-40-5	pos.	0.00003	6.6				Routledge and Sumpter (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	*p* -Nonylphenol	104-40-5	pos.	0.0022					Coldham et al. (1997)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	*p* -Nonylphenol	104-40-5	pos.	92.09					Petit et al. (1997)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	*p* -Nonylphenol	104-40-5	pos.		10				Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	*p* -Nonylphenol	104-40-5	pos.		3				Morito et al. (2001a)
Yeast(*S.cer.*) hER(S)+β-gal(S)	*p* -Nonylphenol benzoate ester		neg.						Odum et al. (1997)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Nonylphenol diethoxylate		pos.	39.64					Petit et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-Nonylphenol diethoxylate		pos.						Routledge and Sumpter (1996)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-Nonylphenol diethoxylate		pos.	0.0000003	666				Routledge and Sumpter (1997)

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Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Nonylphenol heptaethoxylate		pos.	21.34					Petit et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-Nonylphenoxycarboxylic acid		pos.						Routledge and Sumpter (1996)
Yeast(*S.cer.*) hER(S)+β-gal(S)	1-*O* -(Nonylphenyl)-α,β-*D* -glucopyranosiduric acid		neg.				neg.		Moffat et al. (2001)
HeLa hER(T)+CAT(T)	Nordihydroguaiaretic acid	500-38-9	neg.	20					Miksicek (1994)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Nordihydroguaiaretic acid	500-38-9	pos.	<0.000002					Miller et al. (2001)
CHO-K1 hERα(T)+Luc(T)†	Norethindrone	68-22-4	pos.		0.0281				Otsuka Pharmaceutical (2001)
CHO-K1 hERα(T)+Luc(T)†	Norgestrel	6533-00-2	pos.		0.242				Otsuka Pharmaceutical (2001)
CHO-K1 hERα(T)+Luc(T)†	19-Nortestosterone	434-22-0	pos.		0.212				Otsuka Pharmaceutical (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	19-Nortestosterone	434-22-0	neg.						Le Guevel and Pakdel (2001)
MCF-7 hER(E)+CP	Octachlorostyrene	29082-74-4				neg.			Soto et al. (1995)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Octadecyl-3-(3',5'-di-*t* -butyl-4-hydroxyphenyl)propionate	2082-79-3	neg.						Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Octrizole	3147-75-9	neg.						Miller et al. (2001)
MCF-7(Bos) hER(E)+CP	Octyldimethyl-*p* -aminobenzoic acid	21245-02-3		51.77	2.63	pos.			Schlumpf et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	*n* -Octyl gallate	1034-01-1	neg.						Miller et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Octyl isodecyl phthalate	1330-96-7	neg.						Harris et al. (1997)
MCF-7(Bos) hER(E)+CP	Octyl methoxycinnamate	5466-77-3		61.9	2.37	pos.			Schlumpf et al. (2001)
BG-1 hER(E)+Luc(T)	Octylphenol	27193-28-8	pos.	14					Rogers and Denison (2000)
MCF-7 hER(E)+CP	Octylphenol	27193-28-8				pos.			Dodge et al. (1996)
MCF-7 hER(E)+Luc(T)+β-gal(T)	Octylphenol	27193-28-8	pos.						Jobling et al. (1995)

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Yeast(*S.cer.* BJ2407) hER(S)+β-gal(S)	Octylphenol	27193-28-8	pos.	0.001	0.2				Arnold et al. (1996)
ZR-75 hER(E)+CP	Octylphenol	27193-28-8				pos.			Jobling et al. (1995)
HEK293 hERα(T)+Luc(T)+β-gal(T)	4-Octylphenol	1806-26-4	pos.	61					Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	4-Octylphenol	1806-26-4	pos.	57					Kuiper et al. (1998)
MCF-7 hER(E)+CP	4-Octylphenol	1806-26-4				pos.	pos.		Jones et al. (1998)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	4-Octylphenol	1806-26-4	pos.	0.003					Coldham et al. (1997)
CHO-K1 hERα(T)+Luc(T)†	4-*tert* -Octylphenol	140-66-9	neg.		0				Otsuka Pharmaceutical (2001)
HEK293 hERα(T)+Luc(T)+β-gal(T)	4-*tert* -Octylphenol	140-66-9	pos.	70					Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	4-*tert* -Octylphenol	140-66-9	pos.	51					Kuiper et al. (1998)
MCF-7 hER(E)+CP	4-*tert* -Octylphenol	140-66-9				neg.			Jones et al. (1998)
MCF-7 hER(E)+CP	4-*tert* -Octylphenol	140-66-9				pos.			Korner et al. (1998)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	4-*tert* -Octylphenol	140-66-9	neg.						Beresford et al. (2000)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	4-*tert* -Octylphenol	140-66-9	pos.	0.0002					Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	4-*tert* -Octylphenol	140-66-9	pos.	0.0000014	14				Vinggaard et al. (2000)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	4-*tert* -Octylphenol	140-66-9	pos.						Yoshihara et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)+S9*	4-*tert* -Octylphenol	140-66-9	pos.						Yoshihara et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-*tert* -Octylphenol	140-66-9	pos.	0.00004	0.15		neg.		Moffat et al. (2001)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-*tert* -Octylphenol	140-66-9	pos.	0.003	0.01				Routledge and Sumpter (1996)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-*tert* -Octylphenol	140-66-9	pos.	0.0016	0.05				Routledge and Sumpter (1997)

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Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	4-*tert* -Octylphenol	140-66-9	pos.	0.00036					Coldham et al. (1997)
MCF-7 hER(E)+CP	5-Octylphenol					pos.			Soto et al. (1995)
Yeast(*S.cer.*) hER(S)+β-gal(S)	1-*O* -(Octylphenyl)-α,β-*D* -glucopyranosiduronic acid		neg.				neg.		Moffat et al. (2001)
T47D hER(E)+Luc(S)	Org 2058	24320-06-7		-	0				Legler et al. (1999)
Ishikawa hER(T)+Luc(T)+β-gal(T)	Oxamyl	23135-22-0	neg.	0.07			pos.	39%	Klotz et al. (1997)
MCF-7 hER(E)+Luc(T)+β-gal(T)	Oxamyl	23135-22-0		0.07				29%	Klotz et al. (1997)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Paraquat	4685-14-7	pos.	17.99					Petit et al. (1997)
Ishikawa hER(T)+Luc(T)+β-gal(T)	Parathion	56-38-2	neg.	0.03			pos.	35%	Klotz et al. (1997)
MCF-7 hER(E)+CP	Parathion	56-38-2				neg.			Soto et al. (1995)
MCF-7 hER(E)+Luc(T)+β-gal(T)	Parathion	56-38-2		0.03				45%	Klotz et al. (1997)
BG-1 hER(E)+Luc(T)	2,2',3,5',6-Pentachlorobiphenyl	38379-99-6	pos.	17					Rogers and Denison (2000)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	2,2',4,6,6'-Pentachlorobiphenyl	56558-16-8		45			neg.		Fielden et al. (1997)
MCF-7 hER(E)+CP	2,2',4,6,6'-Pentachlorobiphenyl	56558-16-8				pos.	neg.		Fielden et al. (1997)
MCF-7(McGrath) hER(E)+CP	2,3,3',4,4'-Pentachlorobiphenyl	32598-14-4				neg.			Nakagawa and Suzuki (2001)
ZR-75 hER(E)+CP	2,3,3',4,4'-Pentachlorobiphenyl	32598-14-4							Nakagawa and Suzuki (2001)
MCF-7 hER(E)+CP	2,3,3',4,5-Pentachlorobiphenyl	70424-69-0				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP	2,3,4,5,6-Pentachlorobiphenyl	18259-05-7				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP(F)	3,3',4,4',5-Pentachlorobiphenyl	57465-28-8					pos.		Gierthy et al. (1997)
MCF-7(McGrath) hER(E)+CP	3,3',4,4',5-Pentachlorobiphenyl	57465-28-8				neg.			Nakagawa and Suzuki (2001)
ZR-75 hER(E)+CP	3,3',4,4',5-Pentachlorobiphenyl	57465-28-8							Nakagawa and Suzuki (2001)

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HeLa hERαdef(S)+Luc(S)	2,2',3',4',5'-Pentachloro-4-biphenylol	150304-12-4		11			pos.	18	Connor et al. (1997)
MCF-7 hER(T)+CAT(T)	2,2',3',4',5'-Pentachloro-4-biphenylol	150304-12-4	pos.	222			neg.	98.7	Connor et al. (1997)
HeLa hERαdef(S)+Luc(S)	2,2',3',4',6'-Pentachloro-4-biphenylol	150304-10-2	pos.	21			pos.	49	Connor et al. (1997)
MCF-7 hER(T)+CAT(T)	2,2',3',4',6'-Pentachloro-4-biphenylol	150304-10-2	pos.	175			neg.	85.3	Connor et al. (1997)
HeLa hERαdef(S)+Luc(S)	2,2',3',5',6'-Pentachloro-4-biphenylol	150304-11-3	neg.	9			pos.	34	Connor et al. (1997)
MCF-7 hER(T)+CAT(T)	2,2',3',5',6'-Pentachloro-4-biphenylol	150304-11-3	neg.	82			pos.	73.5	Connor et al. (1997)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	2,2',4,6,6'-Pentachloro-4-biphenylol			23			neg.		Fielden et al. (1997)
MCF-7 hER(E)+CP	2,2',4,6,6'-Pentachloro-4-biphenylol					pos.	neg.		Fielden et al. (1997)
MCF-7 hER(E)+Luc(S)	2',3,3',4,4'-Pentachloro-2-biphenylol	150975-80-7			>4.5		pos.		Kramer et al. (1997)
HEK293 hERα(T)+Luc(T)+β-gal(T)	2',3,3',4',5'-Pentachloro-4-biphenylol	192190-09-3	neg.	1			neg.		Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	2',3,3',4',5'-Pentachloro-4-biphenylol	192190-09-3	neg.	1			neg.		Kuiper et al. (1998)
HeLa hERαdef(S)+Luc(S)	2',3,3',4',5'-Pentachloro-4-biphenylol	192190-09-3	neg.	11			pos.	45	Connor et al. (1997)
HeLa hERαdef(S)+Luc(S)	2,3,3',4',5-Pentachloro-4-biphenylol	152969-11-4	neg.	0			pos.		Moore et al. (1997)
HeLa hERαdef(S)+Luc(S)	2',3,3',4',5-Pentachloro-4-biphenylol	149589-55-9	neg.	4			pos.		Moore et al. (1997)
MCF-7 hER(E)+CP	2',3,3',4',5-Pentachloro-4-biphenylol	149589-55-9				neg.	pos.		Moore et al. (1997)
MCF-7 hER(E)+Luc(S)	2,3,3',4',5-Pentachloro-4-biphenylol	152969-11-4			4		pos.		Kramer et al. (1997)
MCF-7 hER(E)+Luc(S)	2',3,3',4',5-Pentachloro-4-biphenylol	149589-55-9			>4.7		pos.		Kramer et al. (1997)
MCF-7 hER(T)+CAT(T)	2',3,3',4',5'-Pentachloro-4-biphenylol	192190-09-3	neg.	28			pos.	50	Connor et al. (1997)
HeLa hERαdef(S)+Luc(S)	2',3,3',4',6'-Pentachloro-4-biphenylol	192190-10-6	pos.	24			neg.	76	Connor et al. (1997)
MCF-7 hER(T)+CAT(T)	2',3,3',4',6'-Pentachloro-4-biphenylol	192190-10-6	neg.	39			pos.	41.1	Connor et al. (1997)
HeLa hERαdef(S)+Luc(S)	2',3,3',5',6'-Pentachloro-4-biphenylol	189578-02-7	neg.	12			pos.	34	Connor et al. (1997)
MCF-7 hER(T)+CAT(T)	2',3,3',5',6'-Pentachloro-4-biphenylol	189578-02-7	neg.	30			pos.	54.6	Connor et al. (1997)
MCF-7 hER(E)+Luc(S)	2',3',4,4',5-Pentachloro-3-biphenylol	150975-81-8			>4.5		pos.		Kramer et al. (1997)

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MCF-7 hER(E)+Luc(S)	2,3',4,4',5-Pentachloro-3-biphenylol	170946-11-9			4.3		pos.		Kramer et al. (1997)
MCF-7 hER(E)+CP	2',3',4',5,5'-Pentachloro-2-biphenylol	67651-36-9				neg.			Soto et al. (1995)
MCF-7 hER(E)+Luc(S)	2',3,4',5,5'-Pentachloro-4-biphenylol	149589-56-0			4.5		pos.		Kramer et al. (1997)
MCF-7 hER(E)+Luc(S)	3,3',4',5,5'-Pentachloro-4-biphenylol	130689-92-8			>4.5		pos.		Kramer et al. (1997)
MCF-7(E3) hER(E)+CP	Pentachlorophenol	87-86-5				neg.			Vinggaard et al. (1999)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Pentachlorophenol	87-86-5	pos.	12.1					Petit et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-*n* -Pentylphenol	14938-35-3	pos.	0.00002	10				Routledge and Sumpter (1997)
HeLa hERα(T)+Luc(T)	Permethrin	52645-53-1	neg.	9.6					Sumida et al. (2001)
MCF-7 hER(E)+CP	Permethrin	52645-53-1				pos.			Go et al. (1999)
BG-1 hER(E)+Luc(S)	Perylene	198-55-0	neg.						Xenobiotic Detection Systems (2001)
MCF-7 hER(E)+CP(F)	Phenanthrene	85-01-8				neg.	neg.		Arcaro et al. (1999b)
Yeast(*S.cer.* ER) hER(S)+β-gal(S)	Phenanthrene	85-01-8	neg.				neg.	99	Tran et al. (1996)
Yeast(*S.cer.* ER179C) hER(S)+β-gal(S)	Phenanthrene	85-01-8					neg.		Tran et al. (1996)
CHO-K1 hERα(T)+Luc(T)†	Phenobarbital, sodium salt	57-30-7	neg.		0				Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Phenol	108-95-2				neg.			Soto et al. (1995)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Phenol	108-95-2	neg.						Miller et al. (2001)
HEK293+hERα(S)+Luc(S)	4-Phenoxyphenol	831-82-3	pos.						Meerts et al. (2001)
HEK293+hERα(S)+Luc(S)	4-Phenoxyphenol	831-82-3	pos.						Meerts et al. (2001)
T47D hER(E)+Luc(S)	4-Phenoxyphenol	831-82-3	pos.		0.0000058		neg.		Meerts et al. (2001)
HEC-1 hERβ(T)+CAT(T)+β-gal(T)	1-Phenyl-3,5-*p* -hydroxyphenyl-4-ethylpyrazole			0.1	0.1				Sun et al. (1999)
BG-1 hER(E)+Luc(S)	2-Phenyl indole	948-65-2	pos.						Xenobiotic Detection Systems (2001)

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Yeast(*S.cer.*) hERα(S)+β-gal(S)	Phenyl salicylate	118-55-8	pos.	0.000003					Miller et al. (2001)
BG-1 hER(E)+Luc(S)	4-Phenyl toluene	644-08-6	pos.						Xenobiotic Detection Systems (2001)
HEK293 hERα(T)+Luc(T)+β-gal(T)	Phloretin	60-82-2	pos.	49			neg.		Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	Phloretin	60-82-2	pos.	10			neg.		Kuiper et al. (1998)
HeLa hER(T)+CAT(T)	Phloretin	60-82-2	pos.	18.9					Miksicek (1993)
HeLa hER(T)+CAT(T)	Phloretin	60-82-2	pos.	98.75	0.3				Miksicek (1994)
MCF-7(M) hER(E)+Luc(T)+β-gal(T)	Phloretin	60-82-2	pos.	40			pos.		Collins-Burow et al. (2000)
MCF-7 hER(E)+CP	Piceatannol	10083-24-6				neg.			Mellanen et al. (1996)
MCF-7 hER(E)+CP	Picloram	1918-02-1				neg.			Soto et al. (1995)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Picloram	1918-02-1	pos.	14.43					Petit et al. (1997)
MCF-7 hER(E)+CP	Pinosylvin	22139-77-1				pos.			Mellanen et al. (1996)
HeLa hER(T)+CAT(T)	Podocarpic acid	5947-49-9	pos.	33.75					Miksicek (1994)
T47D hER(E)+Luc(S)	Polybrominated diphenyl ether 15		neg.				neg.		Meerts et al. (2001)
T47D hER(E)+Luc(S)	Polybrominated diphenyl ether 28		neg.				neg.		Meerts et al. (2001)
HEK293+hERα(S)+Luc(S)	Polybrominated diphenyl ether 30		pos.						Meerts et al. (2001)
HEK293+hERα(S)+Luc(S)	Polybrominated diphenyl ether 30		weak						Meerts et al. (2001)
T47D hER(E)+Luc(S)	Polybrominated diphenyl ether 30		pos.		3.4		neg.		Meerts et al. (2001)
T47D hER(E)+Luc(S)	Polybrominated diphenyl ether 32		pos.		5.1		neg.		Meerts et al. (2001)
T47D hER(E)+Luc(S)	Polybrominated diphenyl ether 47		neg.				neg.		Meerts et al. (2001)
T47D hER(E)+Luc(S)	Polybrominated diphenyl ether 51		pos.		3.1		neg.		Meerts et al. (2001)
T47D hER(E)+Luc(S)	Polybrominated diphenyl ether 71	32534-81-9	pos.		7.3		neg.		Meerts et al. (2001)
T47D hER(E)+Luc(S)	Polybrominated diphenyl ether 75		pos.		2.9		neg.		Meerts et al. (2001)

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T47D hER(E)+Luc(S)	Polybrominated diphenyl ether 77		neg.				neg.		Meerts et al. (2001)
T47D hER(E)+Luc(S)	Polybrominated diphenyl ether 85		neg.				neg.		Meerts et al. (2001)
T47D hER(E)+Luc(S)	Polybrominated diphenyl ether 99		neg.				neg.		Meerts et al. (2001)
HEK293+hERα(S)+Luc(S)	Polybrominated diphenyl ether 100		neg.						Meerts et al. (2001)
HEK293+hERα(S)+Luc(S)	Polybrominated diphenyl ether 100		pos.						Meerts et al. (2001)
T47D hER(E)+Luc(S)	Polybrominated diphenyl ether 100		pos.		2.5		neg.		Meerts et al. (2001)
T47D hER(E)+Luc(S)	Polybrominated diphenyl ether 119		pos.		3.9		neg.		Meerts et al. (2001)
T47D hER(E)+Luc(S)	Polybrominated diphenyl ether 138		neg.				neg.		Meerts et al. (2001)
T47D hER(E)+Luc(S)	Polybrominated diphenyl ether 153		neg.				pos.	3.1	Meerts et al. (2001)
T47D hER(E)+Luc(S)	Polybrominated diphenyl ether 166		neg.				pos.	0.8	Meerts et al. (2001)
T47D hER(E)+Luc(S)	Polybrominated diphenyl ether 190		neg.				pos.	1	Meerts et al. (2001)
MCF-7(E3) hER(E)+CP	Prochloraz	67747-09-5				neg.			Vinggaard et al. (1999)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Prochloraz	67747-09-5	pos.	13.75					Petit et al. (1997)
CHO-K1 hERα(T)+Luc(T)†	Procymidone	32809-16-8	neg.		0				Otsuka Pharmaceutical (2001)
BG-1 hER(E)+Luc(T)	Progesterone	57-83-0	neg.	0					Rogers and Denison (2000)
CHO-K1 hERα(T)+Luc(T)†	Progesterone	57-83-0	neg.		0				Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Progesterone	57-83-0				neg.	neg.		Jones et al. (1998)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Progesterone	57-83-0	neg.						Routledge and Sumpter (1996)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Progesterone	57-83-0	neg.	0					Coldham et al. (1997)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Progesterone	57-83-0	pos.	14					Petit et al. (1999)
Yeast(*S.cer.*) hER(S)+β-gal(S)	17α-Progesterone	2000-66-0	neg.						Routledge and Sumpter (1996)

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Yeast(*S.cer.*) hER(S)+β-gal(S)	17α,20β-Progesterone		neg.						Routledge and Sumpter (1996)
Yeast(*S.cer.* CYT10-5d) hER(S)+β-gal(S)	Promegestone	34184-77-5	neg.	0					Chen et al. (1997)
MCF-7 hER(E)+CP	Propazine	139-40-2				neg.			Soto et al. (1995)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Propham	122-42-9	pos.	17.64					Petit et al. (1997)
MCF-7(E3) hER(E)+CP	Propioconazole	60207-90-1				neg.			Vinggaard et al. (1999)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Propoxylated 4-nonylphenol		neg.	0					Routledge and Sumpter (1997)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	*n* -Propyl gallate	121-79-9	neg.						Miller et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Propylparaben	94-13-3	pos.	0.00003					Miller et al. (2001)
MCF-7 hER(E)+CP	4-Propylphenol	645-56-7				neg.			Soto et al. (1995)
Yeast(*S.cer.*) hER(S)+β-gal(S)	4-Propylphenol	645-56-7	pos.	0.00000005	4000				Routledge and Sumpter (1997)
CHO-K1 hERα(T)+Luc(T)†	Propylthiourea	927-67-3	neg.		0				Otsuka Pharmaceutical (2001)
HEC-1 hERα(T)+CAT(T)+β-gal(T)	4-Propyl-1,3,5-tris(4-hydroxyphenyl)pyrazole		pos.		0.0002				Kraichely et al. (2000)
HEC-1 hERα(T)+CAT(T)+β-gal(T)	4-Propyl-1,3,5-tris(4-hydroxyphenyl)pyrazole			2	0.001				Sun et al. (1999)
HEC-1 hERβ(T)+CAT(T)+β-gal(T)	4-Propyl-1,3,5-tris(4-hydroxyphenyl)pyrazole		neg.				weak		Kraichely et al. (2000)
MCF-7(E3) hER(E)+CP	Propyzamide	23950-58-5				neg.			Vinggaard et al. (1999)
MCF-7 hER(E)+CP	Pseudodiethylstilbestrol	39011-86-4				pos.			Soto et al. (1995)
MCF-7 hER(E)+CP	Pseudodiethylstilbestrol-e	81493-97-2				pos.			Soto et al. (1995)
MCF-7 hER(E)+CP	Pseudodiethylstilbestrol-z	85546-05-0				pos.			Soto et al. (1995)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Purpurogallin	569-77-7	neg.						Miller et al. (2001)

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BG-1 hER(E)+Luc(S)	Pyrene	129-00-0	pos.						Xenobiotic Detection Systems (2001)
MCF-7 hER(E)+CP(F)	Pyrene	129-00-0				neg.	neg.		Arcaro et al. (1999b)
CHO-K1 hERα(T)+Luc(T)†	Pyrethrins	8003-34-7	neg.						Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Pyrethrins	8003-34-7				neg.			Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Pyrethrins	8003-34-7				neg.			Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Pyrethrins	8003-34-7				neg.			Soto et al. (1995)
HEK293 hERα(T)+Luc(T)+β-gal(T)	Quercetin	117-39-5	pos.	3			neg.		Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	Quercetin	117-39-5	pos.	2			neg.		Kuiper et al. (1998)
MCF-7 hER(E)+CP	Quercetin	117-39-5				neg.	pos.		Miodini et al. (1999)
MCF-7(E3) hER(E)+CP	Quintozen	82-68-8				neg.			Vinggaard et al. (1999)
COS-1 mERα(T)+Lucb(T)	Raloxifene	84449-90-1	neg.				pos.		Tremblay et al. (1998)
COS-1 mERα(T)+Luctk(T)	Raloxifene	84449-90-1	neg.				pos.		Tremblay et al. (1998)
COS-1 mERβ(T)+Lucb(T)	Raloxifene	84449-90-1	neg.				pos.		Tremblay et al. (1998)
COS-1 mERβ(T)+Luctk(T)	Raloxifene	84449-90-1	neg.				pos.		Tremblay et al. (1998)
HepG2 hERα(T)+Luc(T)+β-gal(T)	Raloxifene	84449-90-1	neg.				pos.		Gould et al. (1998)
MCF-7 hER(E)+CP(F)	Raloxifene	84449-90-1				neg.	pos.		Arcaro et al. (1998)
MCF-7 hER(E)+CP(F)	Raloxifene	84449-90-1					pos.		Arcaro et al. (1999a)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Resorcinol monobenzoate	136-36-7	pos.	0.00001					Miller et al. (2001)
MCF-7 hER(E)+CP	Resveratrol	501-36-0				neg.			Mellanen et al. (1996)
BG-1 hER(E)+Luc(T)	*trans* -Retinoic acid	302-79-4	neg.	0					Rogers and Denison (2000)
T47D hER(E)+Luc(S)	*trans* -Retinoic acid	302-79-4	neg.		0				Legler et al. (1999)

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CHO-K1 hERα(T)+Luc(T)†	Rimsulfuron	122931-48-0	neg.		0				Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Rotenone	83-79-4				neg.			Soto et al. (1995)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Salicyclic acid	69-72-7	neg.						Miller et al. (2001)
HeLa hER(T)+CAT(T)	Sarsasapogenin	126-19-2	neg.	13.75					Miksicek (1994)
BG-1 hER(E)+Luc(S)	Silvex	93-72-1	neg.						Xenobiotic Detection Systems (2001)
HeLa hER(S)+Luc(S)	Simazine	122-34-9	pos.		3				Balaguer et al. (1996)
MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	Simazine	122-34-9	neg.	0			neg.		Connor et al. (1996)
MCF-7 hER(E)+CP	Simazine	122-34-9				neg.			Soto et al. (1995)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Sissotorin	5928-26-7	neg.				neg.		Morito et al. (2001b)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Sissotorin	5928-26-7	pos.				neg.		Morito et al. (2001b)
MCF-7 hER(E)+CP	β-Sitostanol	83-45-4				pos.			Mellanen et al. (1996)
CHO-K1 hERα(T)+Luc(T)†	β-Sitosterol	83-46-5	neg.		0				Otsuka Pharmaceutical (2001)
HeLa hER(T)+CAT(T)	β-Sitosterol	83-46-5	neg.	16.25					Miksicek (1994)
MCF-7 hER(E)+CP	β-Sitosterol	83-46-5				pos.			Mellanen et al. (1996)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	β-Sitosterol	83-46-5	pos.	0.000005	49.2				Gaido et al. (1997)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Sodium lignosulfonate	8061-51-6	neg.						Routledge and Sumpter (1996)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Sodium *n* -nonyl sulfate	1072-15-7	neg.						Routledge and Sumpter (1996)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Sodium *n* -octyl sulfate	142-31-4	neg.						Routledge and Sumpter (1996)
T47D hER(E)+CP	Spruce Lignan	9005-53-2				neg.			Mellanen et al. (1996)
MCF-7 hER(E)+Luc(T)	*trans* -Stilbene	103-30-0	pos.	48					Sumida et al. (2001)

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HeLa hERα(T)+Luc(T)+S9	*trans* -Stilbene	103-30-0	pos.	155.6					Sumida et al. (2001)
MCF-7 hER(E)+CP	Styrene	100-42-5				neg.			Soto et al. (1995)
HeLa hERα(T)+Luc(T)	Sumithrin	26002-80-2	neg.	7.4					Sumida et al. (2001)
MCF-7 hER(E)+CP	Sumithrin	26002-80-2				pos.			Go et al. (1999)
BG-1 hER(E)+Luc(T)	Tamoxifen	10540-29-1	neg.	0			pos.		Rogers and Denison (2000)
CHO-K1 hERα(T)+Luc(T)†	Tamoxifen	10540-29-1	neg.		0		pos.		Otsuka Pharmaceutical (2001)
HEK-293 hERα(T)+Luc(T)	Tamoxifen	10540-29-1	pos.	8.7			pos.	8.1	Collins-Burow et al. (2000)
HEK-293 hERα(T)+Luc(T)	Tamoxifen	10540-29-1	pos.	8.9			pos.	15.6	Collins-Burow et al. (2000)
HEK293 hERα(T)+Luc(T)+β-gal(T)	Tamoxifen	10540-29-1	pos.	6			pos.		Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	Tamoxifen	10540-29-1	pos.	2			neg.		Kuiper et al. (1998)
HeLa mER(T)+CAT(T)*	Tamoxifen	10540-29-1	pos.	36 (1 µM)					Shelby et al. (1996)
MCF-7 hER(E)+CP	Tamoxifen	10540-29-1				neg.			Jones et al. (1998)
MCF-7 hER(E)+CP	Tamoxifen	10540-29-1					pos.		Korner et al. (1998)
MCF-7(M) hER(E)+Luc(T)+β-gal(T)	Tamoxifen	10540-29-1					pos.		Collins-Burow et al. (2000)
MCF-7(M) hER(E)+CP	Tamoxifen	10540-29-1				neg.	pos.		Collins-Burow et al. (2000)
T47D hER(E)+Luc(S)	Tamoxifen	10540-29-1					pos.		Legler et al. (1999)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Tamoxifen	10540-29-1					pos.		Routledge and Sumpter (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Tamoxifen	10540-29-1	pos.	0.0047					Coldham et al. (1997)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Tamoxifen	10540-29-1	neg.						Morito et al. (2001a)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Tamoxifen	10540-29-1	neg.						Morito et al. (2001a)

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MCF-7 hER(E)+CP	*cis* -Tamoxifen	13002-65-8				pos.			Soto et al. (1995)
Yeast(*S.cer* .939) hER(S)+β-gal(S)	Tamoxifen citrate	54965-24-1	neg.	0			pos.	36.4	Chen et al. (1997)
MCF-7 hER(E)+CP	Tamoxifen metabolite E	68684-63-9				pos.			Soto et al. (1995)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Tectoridin	611-40-5	neg.				neg.		Morito et al. (2001b)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Tectoridin	611-40-5	pos.				neg.		Morito et al. (2001b)
Yeast(*S.cer.* Y190) hERα(S)+β-gal(S)	Tectorigenin	548-77-6	pos.		2		neg.		Morito et al. (2001b)
Yeast(*S.cer.* Y190) hERβ(S)+β-gal(S)	Tectorigenin	548-77-6	pos.		6.2		weak		Morito et al. (2001b)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Terbacil	5902-51-2	pos.	17.87					Petit et al. (1997)
BG-1 hER(E)+Luc(T)	Testosterone	58-22-0	neg.	0					Rogers and Denison (2000)
CHO-K1 hERα(T)+Luc(T)†	Testosterone	58-22-0	neg.		0				Otsuka Pharmaceutical (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Testosterone	58-22-0	pos.	0.0001					Beresford et al. (2000)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Testosterone	58-22-0	neg.						Routledge and Sumpter (1996)
Yeast(*S.cer.*) hER(S)+β-gal(S)	Testosterone	58-22-0	neg.	0					Routledge and Sumpter (1997)
Yeast(*S.cer* .939) hER(S)+β-gal(S)	Testosterone	58-22-0	pos.	0.014					Chen et al. (1997)
Yeast(*S.cer.* BJ2407) hER(S)+β-gal(S)	Testosterone	58-22-0	neg.						Arnold et al. (1996)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Testosterone	58-22-0	pos.	0.001					Coldham et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Testosterone	58-22-0	pos.	0.000005	50.9				Gaido et al. (1997)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Testosterone	58-22-0	neg.						Le Guevel and Pakdel (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Testosterone	58-22-0	pos.	14					Petit et al. (1999)

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MCF-7 hER(E)+CP	Tetrabromobisphenol A	79-94-7				pos.			Korner et al. (1998)
T47D hER(E)+Luc(S)	Tetrabromobisphenol A	79-94-7	neg.						Meerts et al. (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Tetrabromobisphenol A	79-94-7	neg.						Miller et al. (2001)
MCF-7 hER(E)+CP	2,2',4,5-Tetrachlorobiphenyl	70362-47-9				pos.			Soto et al. (1995)
MCF-7(McGrath) hER(E)+CP	2,2',5,5'-Tetrachlorobiphenyl	35693-99-3				neg.	neg.		Nakagawa and Suzuki (2001)
T47D hER(E)+CAT(T)	2,2',5,5'-Tetrachlorobiphenyl	35693-99-3	neg.	0					Nakagawa and Suzuki (2001)
ZR-75 hER(E)+CP	2,2',5,5'-Tetrachlorobiphenyl	35693-99-3				neg.	neg.		Nakagawa and Suzuki (2001)
MCF-7 hER(E)+CP(F)	2,2',6,6'-Tetrachlorobiphenyl	15968-05-5			2	pos.	neg.		Arcaro et al. (1999a)
BG-1 hER(E)+Luc(T)	2,3',4,4'-Tetrachlorobiphenyl	32598-10-0	pos.	9					Rogers and Denison (2000)
MCF-7 hER(E)+CP	2,3,4,4'-Tetrachlorobiphenyl	33025-41-1				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP	2,3,4,5-Tetrachlorobiphenyl	33284-53-6				neg.			Soto et al. (1994)
MCF-7 hER(E)+CP	2,3,4,5-Tetrachlorobiphenyl	33284-53-6				pos.			Soto et al. (1995)
MCF-7 hER(E)+CP	2,3,5,6-Tetrachlorobiphenyl	33284-54-7				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP	2,4,4',6-Tetrachlorobiphenyl	32598-12-2				pos.			Soto et al. (1995)
BG-1 hER(E)+Luc(T)	3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	neg.	0					Rogers and Denison (2000)
MCF-7(McGrath) hER(E)+CP	3,3',5,5'-Tetrachlorobiphenyl	33284-52-5				pos.	neg.		Nakagawa and Suzuki (2001)
T47D hER(E)+CAT(T)	3,3',5,5'-Tetrachlorobiphenyl	33284-52-5	pos.	0.8					Nakagawa and Suzuki (2001)
ZR-75 hER(E)+CP	3,3',5,5'-Tetrachlorobiphenyl	33284-52-5				weak	neg.		Nakagawa and Suzuki (2001)
MCF-7 hER(E)+Luc(S)	3,3',5,5'-Tetrachloro-4,4'-biphenyldiol	13049-13-3			4.3		pos.		Kramer et al. (1997)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	3,3',5,5'-Tetrachloro-4,4'-biphenyldiol	13049-13-3	pos.	0.016					Coldham et al. (1997)
HEK293 hERα(T)+Luc(T)+β-gal(T)	2,2',4',6'-Tetrachloro-4-biphenylol	150304-08-8	pos.	3			neg.		Kuiper et al. (1998)

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HEK293 hERβ(T)+Luc(T)+β-gal(T)	2,2',4',6'-Tetrachloro-4-biphenylol	150304-08-8	pos.	3			neg.		Kuiper et al. (1998)
HeLa hERαdef(S)+Luc(S)	2,2',4',6'-Tetrachloro-4-biphenylol	150304-08-8	pos.	50			pos.	62	Connor et al. (1997)
MCF-7 hER(T)+CAT(T)	2,2',4',6'-Tetrachloro-4-biphenylol	150304-08-8	neg.	69			pos.	63	Connor et al. (1997)
MCF-7 hER(E)+CP(F)	2,2',6,6'-Tetrachloro-4-biphenylol	219952-18-8				pos.			Arcaro et al. (1999a)
MCF-7 hER(E)+CP	2',3',4',5'-Tetrachloro-3-biphenylol	67651-37-0				pos.			Soto et al. (1995)
MCF-7 hER(E)+Luc(S)	2',3',4',5'-Tetrachloro-3-biphenylol	67651-37-0			4.1		pos.		Kramer et al. (1997)
HEK293 hERα(T)+Luc(T)+β-gal(T)	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	pos.	68					Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	pos.	41					Kuiper et al. (1998)
HeLa hERαdef(S)+Luc(S)	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	pos.	74			neg.		Moore et al. (1997)
HepG2 hER(T)+Luc(T)+β-gal(T)	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	pos.	61%					Ramamoorthy et al. (1997b)
MCF-7 hER(E)+CP	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7				pos.			Soto et al. (1995)
MCF-7 hER(E)+CP(F)	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7			0.72		neg.		Arcaro et al. (1998)
MCF-7 hER(E)+Luc(S)	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7			>4.7		pos.		Kramer et al. (1997)
MCF-7 hER(T)+CAT(T)	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7		88%					Ramamoorthy et al. (1997b)
MCF-7 hER(T)+Luc(T)	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7		81%					Ramamoorthy et al. (1997b)
MDA-MB-231 hER(T)+Luc(T)	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	pos.	0.61					Ramamoorthy et al. (1997b)
Yeast(*S.cer.* BJ3503) hER(S)+β-gal(S)	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7		0.000003	0.3				Ramamoorthy et al. (1997b)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	2',3',4',5'-Tetrachloro-4-biphenylol	67651-34-7	pos.	0.82					Coldham et al. (1997)
HeLa hERαdef(S)+Luc(S)	2',3,4',6'-Tetrachloro-4-biphenylol	189578-00-5	pos.	60			pos.	80	Connor et al. (1997)
MCF-7 hER(T)+CAT(T)	2',3,4',6'-Tetrachloro-4-biphenylol	189578-00-5	neg.	33			pos.	54.6	Connor et al. (1997)
HeLa hER(S)+Luc(S)	2,3,7,8-Tetrachlorodibenzo-*p* -dioxin	1746-01-6	pos.		0.00003				Balaguer et al. (1996)

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MCF-7 hERαdef(T)+Luc(T)+β-gal(T)	2,3,7,8-Tetrachlorodibenzo-*p* -dioxin	1746-01-6							Clemons et al. (1998)
MCF-7 hER(E)+CP	2,3,7,8-Tetrachlorodibenzo-*p* -dioxin	1746-01-6				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP(F)	2,3,7,8-Tetrachlorodibenzo-*p* -dioxin	1746-01-6					pos.		Arcaro et al. (1999a)
MCF-7 hER(E)+CP(F)	2,3,7,8-Tetrachlorodibenzo-*p* -dioxin	1746-01-6				neg.	pos.		Arcaro et al. (1999b)
MCF-7 hER(E)+CP(F)	2,3,7,8-Tetrachlorodibenzo-*p* -dioxin	1746-01-6				neg.	pos.		Gierthy et al. (1997)
T47D hER(E)+Luc(S)	2,3,7,8-Tetrachlorodibenzo-*p* -dioxin	1746-01-6					pos.		Legler et al. (1999)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	2,3,7,8-Tetrachlorodibenzo-*p* -dioxin	1746-01-6	pos.	0.26					Coldham et al. (1997)
MCF-7 hER(E)+CP	Tetrachloroethylene	127-18-4				neg.			Soto et al. (1995)
MCF-7(E3) hER(E)+CP	Tetrachlorvinphos	22248-79-9				neg.			Vinggaard et al. (1999)
MCF-7(E3) hER(E)+CP	Tetradifon	116-29-0				neg.			Vinggaard et al. (1999)
HeLa hER(T)+CAT(T)	2',4',4,6'-Tetrahydroxychalcone	73692-50-9	pos.	85					Miksicek (1993)
MCF-7 hER(E)+CP	Thiram	137-26-8				neg.			Soto et al. (1995)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Thymol	89-83-8	neg.						Miller et al. (2001)
CHO-K1 hERα(T)+Luc(T)†	Toxaphene	8001-35-2	neg.		0				Otsuka Pharmaceutical (2001)
ELT-3 hER(T)+Luc(T)+β-gal(T)	Toxaphene	8001-35-2	pos.	10.06					Hodges et al. (2000)
MCF-7 hER(E)+CP	Toxaphene	8001-35-2				pos.			Ramamoorthy et al. (1997a)
MCF-7 hER(E)+CP	Toxaphene	8001-35-2				pos.			Soto et al. (1994)
MCF-7 hER(E)+CP	Toxaphene	8001-35-2				pos.			Soto et al. (1995)
MCF-7 hER(T)+CAT(T)	Toxaphene	8001-35-2		4.8		neg.			Ramamoorthy et al. (1997a)
Yeast(*S.cer.* BJ2168) mER(S)+β-gal(S)	Toxaphene	8001-35-2	pos.	10x (25 µM)*					Ramamoorthy et al. (1997a)
Yeast(*S.cer.* BJ2168) mER(S)+β-gal(S)	Toxaphene	8001-35-2	pos.	0.6x (100 µM)*					Ramamoorthy et al. (1997a)

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CHO-K1 hERα(T)+Luc(T)†	Tralomethrin	66841-25-6	pos.						Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Tralomethrin	66841-25-6				pos.			Otsuka Pharmaceutical (2001)
MCF-7 hER(E)+CP	Tralomethrin	66841-25-6				pos.			Otsuka Pharmaceutical (2001)
HEC-1 hERα(T)+CAT(T)+β-gal(T)	*trans* -3b		pos.	95			neg.		Meyers et al. (1999)
HEC-1 hERβ(T)+CAT(T)+β-gal(T)	*trans* -3b		pos.	68			pos.		Meyers et al. (1999)
HEC-1 hERα(T)+CAT(T)+β-gal(T)	*trans* -3c		pos.	81			neg.		Meyers et al. (1999)
HEC-1 hERβ(T)+CAT(T)+β-gal(T)	*trans* -3c		neg.	10			pos.		Meyers et al. (1999)
MCF-7 hER(E)+CP	Transforming Growth Factor α (TGF-α)					pos.			Miodini et al. (1999)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	β-Trenbolone	10161-33-8	neg.						Le Guevel and Pakdel (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Trenbolone acetate	10161-34-9	neg.						Le Guevel and Pakdel (2001)
MCF-7(E3) hER(E)+CP	Triadimefon	43121-43-3				pos.			Vinggaard et al. (1999)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Triadimefon	43121-43-3	pos.	17.24					Petit et al. (1997)
MCF-7(E3) hER(E)+CP	Triadimenol	55219-65-3				pos.			Vinggaard et al. (1999)
T47D hER(E)+Luc(S)	3,3',5-Tribromobisphenol A	6386-73-8	neg.						Meerts et al. (2001)
HEK293+hERα(S)+Luc(S)	4-(2,4,6-Tribromophenoxy)phenol		pos.						Meerts et al. (2001)
HEK293+hERα(S)+Luc(S)	4-(2,4,6-Tribromophenoxy)phenol		pos.						Meerts et al. (2001)
T47D hER(E)+Luc(S)	4-(2,4,6-Tribromophenoxy)phenol		neg.		0.0001		neg.		Meerts et al. (2001)
MCF-7 hER(E)+CP(F)	2,2',3-Trichlorobiphenyl	38444-78-9				pos.			Arcaro et al. (1999a)
MCF-7 hER(E)+CP(F)	2,2',6-Trichlorobiphenyl	38444-73-4				pos.			Arcaro et al. (1999a)
MCF-7 hER(E)+CP	2,3,4-Trichlorobiphenyl	55702-46-0				pos.			Soto et al. (1995)

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MCF-7 hER(E)+CP	2,3',5-Trichlorobiphenyl	38444-81-4				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP	2,3,6-Trichlorobiphenyl	55702-45-9				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP(F)	2,3',6-Trichlorobiphenyl	38444-76-7				neg.			Arcaro et al. (1999a)
MCF-7 hER(E)+CP(F)	2,4,6-Trichlorobiphenyl	35693-92-6				pos.	neg.		Gierthy et al. (1997)
MCF-7 hER(E)+CP(F)	3,4',5-Trichlorobiphenyl	38444-88-1				pos.	pos.		Gierthy et al. (1997)
HeLa mER(T)+CAT(T)	2,4,6-Trichloro-3',4'-biphenyldiol		pos.	35.7					Garner et al. (1999)
MCF-7 hER(E)+CP	2,2',5-Trichloro-4-biphenylol					pos.			Soto et al. (1995)
HEK293 hERα(T)+Luc(T)+β-gal(T)	2',4',6'-Trichloro-4-biphenylol	14962-28-8	pos.	77					Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	2',4',6'-Trichloro-4-biphenylol	14962-28-8	pos.	62					Kuiper et al. (1998)
HeLa mER(T)+CAT(T)	2',4',6'-Trichloro-4-biphenylol	14962-28-8	pos.	44.2					Garner et al. (1999)
HepG2 hER(T)+Luc(T)+β-gal(T)	2',4',6'-Trichloro-4-biphenylol	14962-28-8	pos.	70					Ramamoorthy et al. (1997b)
MCF-7 hER(E)+CP	2',4',6'-Trichloro-4-biphenylol	14962-28-8				pos.			Soto et al. (1995)
MCF-7 hER(E)+CP(F)	2',4',6'-Trichloro-4-biphenylol	14962-28-8			0.22		neg.		Arcaro et al. (1998)
MCF-7 hER(E)+CP(F)	2',4',6'-Trichloro-4-biphenylol	14962-28-8				pos.	neg.		Gierthy et al. (1997)
MCF-7 hER(E)+Luc(S)	2',4',6'-Trichloro-4-biphenylol	14962-28-8			>4.7		pos.		Kramer et al. (1997)
MCF-7 hER(T)+CAT(T)	2',4',6'-Trichloro-4-biphenylol	14962-28-8		95%			.		Ramamoorthy et al. (1997b)
MCF-7 hER(T)+Luc(T)	2',4',6'-Trichloro-4-biphenylol	14962-28-8		52%					Ramamoorthy et al. (1997b)
MDA-MB-231 hER(T)+Luc(T)	2',4',6'-Trichloro-4-biphenylol	14962-28-8	pos.	0.82					Ramamoorthy et al. (1997b)
Yeast(*S.cer.* BJ3503) hER(S)+β-gal(S)	2',4',6'-Trichloro-4-biphenylol	14962-28-8		0.00001	0.08				Ramamoorthy et al. (1997b)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	2',4',6'-Trichloro-4-biphenylol	14962-28-8	pos.	1					Coldham et al. (1997)
MCF-7 hER(E)+Luc(S)	3,3',4'-Trichloro-4-biphenylol	124882-64-0			>4.6		pos.		Kramer et al. (1997)
MCF-7 hER(E)+CP(F)	3,4',5-Trichloro-4-biphenylol	4400-06-0				pos.	neg.		Gierthy et al. (1997)

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BG-1 hER(E)+Luc(S)	2,4,5-Trichlorophenoxyacetic acid	93-76-5	pos.						Xenobiotic Detection Systems (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Triethanolamine salicylate	2174-16-5	neg.						Miller et al. (2001)
MCF-7 hER(E)+CP	Trifluralin	1582-09-8				neg.			Soto et al. (1995)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Trifluralin	1582-09-8	pos.	19.32					Petit et al. (1997)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	2,4,5-Trihydroxybutyrophenone	1421-63-2	neg.						Miller et al. (2001)
HepG2 hERα(T)+Luc(T)+β-gal(T)	Trihydroxymethoxychlor		pos.	45			neg.	100	Gaido et al. (2000)
HepG2 hERβ(T)+Luc(T)+β-gal(T)	Trihydroxymethoxychlor		pos.	15			neg.	95µM	Gaido et al. (2000)
BG-1 hER(E)+Luc(T)	3,3',5'-Triiodo-1-thyronine	6893-02-3	neg.	0					Rogers and Denison (2000)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	3,3',5'-Triiodo-1-thyronine	6893-02-3	pos.	18					Petit et al. (1999)
HepG2 hERα(T)+Luc(T)+β-gal(T)	Trimethoxymethoxychlor		pos.	20			neg.	100	Gaido et al. (2000)
HepG2 hERβ(T)+Luc(T)+β-gal(T)	Trimethoxymethoxychlor		pos.	15			neg.	100µM	Gaido et al. (2000)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	1,3,5-Trimethyl-2,4,6-tris(3,5-di-*tert* -butyl-4-hydroxybenzyl)benzene	1709-70-2	neg.						Miller et al. (2001)
CHO-K1 hERα(T)+Luc(T)†	Triphenyltin chloride	639-58-7					pos.		Otsuka Pharmaceutical (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	1,3,5-Tris(4-*tert* -butyl-3-hydroxy-2,6,-dimethylbenzyl)isocyanurate	40601-76-1	neg.						Miller et al. (2001)
MCF-7(MELN41) hER(E)+Luc(S)	Tris-4-(chlorophenyl)methane	27575-78-6	pos.						Lascombe et al. (2000)
Yeast(*S.cer.* YRG-2) hERα(S)+β-gal(S)	Tris-4-(chlorophenyl)methane	27575-78-6	pos.	4x (100 uM)*					Lascombe et al. (2000)
MCF-7(MELN41) hER(E)+Luc(S)	Tris-4-(chlorophenyl)methanol	30100-80-8	pos.						Lascombe et al. (2000)
Yeast(*S.cer.* YRG-2) hERα(S)+β-gal(S)	Tris-4-(chlorophenyl)methanol	30100-80-8	pos.	6x (100 uM)*					Lascombe et al. (2000)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Vanillin	121-33-5	neg.						Miller et al. (2001)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
CHO-K1 hERα(T)+Luc(T)†	Vinclozolin	50471-44-8	neg.		0				Otsuka Pharmaceutical (2001)
Yeast(*S.cer.*) hERα(S)+β-gal(S)	Vitamin E	59-02-9	neg.						Miller et al. (2001)
BG-1 hER(E)+Luc(S)	*p* -Xylene	106-42-3	neg.						Xenobiotic Detection Systems (2001)
CHO-K1 hERα(S)+Luc(S)†	α-Zearalanol	26538-44-3	pos.		0.000131				Otsuka Pharmaceutical (2001)
CHO-K1 hERα(T)+Luc(T)†	α-Zearalanol	26538-44-3	pos.		0.0000783				Otsuka Pharmaceutical (2001)
Ishikawa hER(E)+CP	α-Zearalanol	26538-44-3				pos.			Le Guevel and Pakdel (2001)
MCF-7 hER(E)+CP	α-Zearalanol	26538-44-3				pos.			Soto et al. (1995)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	α-Zearalanol	26538-44-3	pos.	1.3					Coldham et al. (1997)
Yeast(*S.cer.* BJ-ECZ) hER(S)+β-gal(S)	α-Zearalanol	26538-44-3	pos.	1.7	0.04				Le Guevel and Pakdel (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	α-Zearalanol	26538-44-3	pos.	16	0.03				Le Guevel and Pakdel (2001)
Ishikawa hER(E)+CP	β-Zearalanol	42422-68-4				pos.			Le Guevel and Pakdel (2001)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	β-Zearalanol	42422-68-4	pos.	0.46					Coldham et al. (1997)
Yeast(*S.cer.* BJ-ECZ) hER(S)+β-gal(S)	β-Zearalanol	42422-68-4	pos.	0.5	0.16				Le Guevel and Pakdel (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	β-Zearalanol	42422-68-4	pos.	4.4	0.11				Le Guevel and Pakdel (2001)
Ishikawa hER(E)+CP	Zearalanone	5975-78-0				pos.			Le Guevel and Pakdel (2001)
Yeast(*S.cer.* BJ-ECZ) hER(S)+β-gal(S)	Zearalanone	5975-78-0	pos.	0.7	0.11				Le Guevel and Pakdel (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Zearalanone	5975-78-0	pos.	16	0.032				Le Guevel and Pakdel (2001)
Ishikawa hER(E)+CP	α-Zearalenol	36455-72-8				pos.			Le Guevel and Pakdel (2001)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
MCF-7(M) hER(E)+Luc(T)+β-gal(T)	α-Zearalenol	36455-72-8	pos.	50			pos.		Collins-Burow et al. (2000)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	α-Zearalenol	36455-72-8	pos.	8.7					Coldham et al. (1997)
Yeast(*S.cer.* BJ-ECZ) hER(S)+β-gal(S)	α-Zearalenol	36455-72-8	pos.	2.7	0.03				Le Guevel and Pakdel (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	α-Zearalenol	36455-72-8	pos.	43	0.012				Le Guevel and Pakdel (2001)
HeLa hER(T)+CAT(T)	β-Zearalenol	71030-11-0	pos.	59.9					Miksicek (1993)
HeLa hER(T)+CAT(T)	β-Zearalenol	71030-11-0	pos.	102.25	0.015				Miksicek (1994)
Ishikawa hER(E)+CP	β-Zearalenol	71030-11-0				pos.			Le Guevel and Pakdel (2001)
MCF-7(M) hER(E)+Luc(T)+β-gal(T)	β-Zearalenol	71030-11-0	pos.	53			pos.		Collins-Burow et al. (2000)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	β-Zearalenol	71030-11-0	pos.	0.066					Coldham et al. (1997)
Yeast(*S.cer.* BJ-ECZ) hER(S)+β-gal(S)	β-Zearalenol	71030-11-0	pos.	0.2	0.28				Le Guevel and Pakdel (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	β-Zearalenol	71030-11-0	neg.						Le Guevel and Pakdel (2001)
BG-1 hER(E)+Luc(S)	Zearalenone	17924-92-4	pos.	1	0.001				Xenobiotic Detection Systems (2001)
CHO-K1 hERα(T)+Luc(T)†	Zearalenone	17924-92-4	pos.		0.00729				Otsuka Pharmaceutical (2001)
HEK293 hERα(T)+Luc(T)+β-gal(T)	Zearalenone	17924-92-4	pos.	91			neg.		Kuiper et al. (1998)
HEK293 hERβ(T)+Luc(T)+β-gal(T)	Zearalenone	17924-92-4	pos.	27			pos.		Kuiper et al. (1998)
HeLa hER(T)+CAT(T)	Zearalenone	17924-92-4	pos.	99.8					Miksicek (1993)
HeLa hER(T)+CAT(T)	Zearalenone	17924-92-4	pos.	141.25	0.002				Miksicek (1994)
HeLa mER(T)+CAT(T)	Zearalenone	17924-92-4		185		pos.			Makela et al. (1994)
HeLa mER(T)+CAT(T)*	Zearalenone	17924-92-4	pos.	100x (100 nM)					Makela et al. (1994)
Ishikawa hER(E)+CP	Zearalenone	17924-92-4				pos.			Le Guevel and Pakdel (2001)

Assay**	Substance Name	CASRN†	Agonism (Qualitative)††	Agonism (Relative Activity)††	Agonism (EC50 µM)††	Cell Growth††	Antagonism (Qualitative)††	Antagonism (Relative Activity)††	Reference
MCF-7 hER(E)+CP	Zearalenone	17924-92-4				pos.	neg.		Makela et al. (1994)
MCF-7 hER(E)+CP	Zearalenone	17924-92-4				pos.			Soto et al. (1995)
MCF-7(M) hER(E)+Luc(T)+β-gal(T)	Zearalenone	17924-92-4	pos.	75			pos.		Collins-Burow et al. (2000)
Yeast(S.cer. BJ3505) hER(S)+β-gal(S)	Zearalenone	17924-92-4	pos.	0.26					Coldham et al. (1997)
Yeast(*S.cer.* BJ-ECZ) hER(S)+β-gal(S)	Zearalenone	17924-92-4	pos.	0.6	0.13				Le Guevel and Pakdel (2001)
Yeast(*S.cer.* BJ-ECZ) rtER(S)+β-gal(S)	Zearalenone	17924-92-4	pos.	8.8	0.062				Le Guevel and Pakdel (2001)
HeLa mER(T)+CAT(T)*	Zeranol	55331-29-8	pos.	103x (1 nM)					Makela et al. (1994)
MCF-7 hER(E)+CP	Zeranol	55331-29-8				pos.			Dodge et al. (1996)
MCF-7 hER(E)+CP	Zeranol	55331-29-8				pos.	neg.		Makela et al. (1994)
MCF-7 hER(E)+CP	Zineb	12122-67-7				neg.			Soto et al. (1995)
MCF-7 hER(E)+CP	Ziram	137-30-4				neg.			Soto et al. (1995)

[a]wi = a compound that, at 10 µM, induces reporter gene expression at <50% of maximal induction caused by 10 nM E2.

[b]ni = a compound that, at 10 µM, induces reporter gene expression at <10% of maximal induction casued by 10 nM E2.

† Empty cells indicate that no CASRN could be found

†† Empty cells indicate that no information was provided in the publication

* Indicates the concentration at which the fold increase was reported

**There are at least 93 different assays. Variations include different mammalian cell lines [BG-1, CHO, COS-1, ELT-3, HEK293, HEC-1, HeLa, HEPG2, Ishikawa, MCF-7, MCF-7(M), MCF-7(BUS), MCF-7(E3), MCF-7(McGrath), MCF-7(MEL:N411), MDA-MB-231, T47D, ZR75], and different strains of Yeast, *Sacchromyces cerevisiae* abbreviated as *S.cer* ., [.188R1,.939, BJ2168, BJ2407,BJ3505, BJ-ECZ, YT10-5d, ER, ER179C, Y190, YRG-2]. Sources of the ER were from human (hER), mouse (mER), rainbow trout (rtER) and included either an undefined ER or the α or β form of the ER. In a few cases the binding domain (def) was used instead of the entire protein. In the mammalian cells either chloramphenicol acetyltransferase (CAT) or luciferase (Luc) was the enzyme induced by transcriptional activation. In the yeast cells β-galactosidase (β-gal) was the induced enzyme. Plasmids were either transiently (T) or stably (S) transfected. Some cell lines harbored an endogenous ER (E). A plasmid with β-gal was sometimes transfected into the mammalian cells as a measure of transfection. In certain assays, cell prolif

Values in italics have been estimated from a graphical representation of the data

Appendix D2

Substances Tested in the *In Vitro* ER TA Assays

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Appendix E

Responses of Substances Tested in at Least Two Different Types of *In Vitro* ER TA Assays

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Responses of Substances Tested in at Least Two Different Types of *In Vitro* ER TA Assays

Substance	Agonism Assays			Antagonism Assays		
	Mammalian Cell Reporter Gene	Yeast Reporter Gene	Mammalian Cell Proliferation	Mammalian Cell Reporter Gene	Yeast Reporter Gene	Mammalian Cell Proliferation
Acenaphthylene	neg (1)*	neg (1)			neg (2)	
Acrinathrin	neg (1)		neg (2)			
Alachlor	pos (1)	pos (2)	neg (1)			
Androstenedione	neg (1)	neg (2)				
Anthracene	neg (1)	neg (1)	neg (1)		neg (2)	neg (1)
Apigenin	pos (6)		pos (1/2)	pos (3/5)		pos (1)
Aroclor 1221	pos (1)	pos (1)				
Aroclor 1248	neg (1)	pos (1)				
Atrazine	neg (3)	pos (1/2)	neg (1)	neg (1)	neg (1)	
Bendiocarb	neg (2)		neg (1)			
Benz[*a*]anthracene	pos (4)		neg (1)			
Benzo[*b*]fluoranthene		neg (1)	neg (1)		neg (2)	pos (1)
Benzo[*k*]fluoranthene	pos (2/3)	neg (1)	neg (1)		neg (2)	pos (1)
Benzo[*b*]fluorene	pos (2)	neg (1)			pos (1/2)	

Substance	Agonism Assays			Antagonism Assays		
	Mammalian Cell Reporter Gene	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**	**Mammalian Cell Reporter Gene**	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**
Benzo[*ghi*]perylene	neg (1)		neg (1)			
Benzophenone-3		pos (1)	pos (1)			
Benzo[*a*]pyrene	pos (4/5)	neg (1)	neg (1)	neg (2)	pos (1/2)	pos (1)
Biochanin A	pos (5)	pos (2/4)	pos (1)	pos (1/3)	pos (2)	neg (1)
4,4'-Biphenol	pos (2)	pos (2)	pos (1)			
4-Biphenylol		pos (4)	pos (2)			
Bis(2-ethylhexyl)phthalate	pos (1/3)	pos (1/3)	neg (4)			
Bis(4-hydroxyphenyl)ethane	pos (2)			neg (2)		
Bis(4-hydroxyphenyl)methane	pos (2)	pos (1)		neg (2)		
Bisphenol A	pos (15)	pos (14)	pos (8)	neg (2)		
Bisphenol A dimethacrylate	pos (1)		pos (3/4)			
Bisphenol B	pos (2)	pos (2)				
2,2-Bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane (HPTE)	pos (13)	pos (3)		pos (5/8)		
Butylated hydroxyanisole	pos (1)	pos (1)	pos (3/4)			
Butylated hydroxytoluene	pos (1)	pos (1/2)	pos (1/3)			

Substance	Agonism Assays			Antagonism Assays		
	Mammalian Cell Reporter Gene	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**	**Mammalian Cell Reporter Gene**	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**
Butyl benzyl phthalate	pos (3/4)	pos (3)	pos (5/6)			
4-*tert*-Butylphenol		pos (3)	pos (2)			
Carbaryl	neg (2)		neg (1)			
Carbofuran		pos (1)	neg (1)			
Chlordane	pos (1)	pos (2)	neg (1)			
Tris-4-(chlorophenyl)methane	pos (1)	pos (1)				
Tris-4-(chlorophenyl)methanol (Tris-OH)	pos (1)	pos (1)				
Chlorothalonil		pos (1)	neg (1)			
Cholesterol		neg (1)	neg (1)			
Chrysene	pos (2/4)	neg (1)	neg (1)		neg (2)	pos (1)
Clomiphene	neg (1)	pos (1)				
Cortisol	neg (1)	neg (3)				
Coumestrol	pos (7)	pos (4)	pos (4)	neg (2/3)	neg (2)	neg (2)
o-Cresol	neg (1)	neg (1)				
p-Cresol	neg (1)	neg (2)				

Substance	Agonism Assays			Antagonism Assays		
	Mammalian Cell Reporter Gene	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**	**Mammalian Cell Reporter Gene**	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**
Cycloprothrin	pos (1)		neg (2)			
Cyfluthrin	pos (1)		pos (2)			
Cyhalothrin	pos (1)		pos (2)			
Cypermethrin	neg (2)		pos (2)			
2,4-Dichlorophenoxyacetic acid		pos (1)	neg (1)			
Daidzein	pos (5)	pos (3/4)	pos (2)	neg (2)		
Daidzin		pos (1/2)	pos (1)			
o,p'-DDD	pos (1)	pos (4)				
p,p'-DDD	pos (1/2)	pos (1)				
o,p'-DDE	neg (1)	pos (4)	pos (1)			
p,p'-DDE	pos (2/4)	neg (2)	pos (2/3)	neg (2)		
o,p'-DDT	pos (7/8)	pos (10)	pos (3)			
p,p'-DDT	pos (3)	pos (4)	pos (2)			
Deltamethrin	pos (1)	pos (1)	pos (2)			
Dexamethasone	pos (1)	pos (1/4)				
Diamyl phthalate		neg (1)	neg (1)			

Substance	Agonism Assays			Antagonism Assays		
	Mammalian Cell Reporter Gene	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**	**Mammalian Cell Reporter Gene**	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**
Dibenz[*a*,*h*]anthracene	pos (1/2)	neg (1)	neg (1)		pos (2)	pos (1)
Dibutyl phthalate	pos (2/4)	pos (3/4)	pos (4/5)			
2,5-Dichloro-4'-biphenylol	pos (1)	pos (1)	pos (2)			
Dicofol	neg (1)	pos (1)	neg (1)			
Dieldrin	pos (3)	pos (4)	pos (2/4)		neg (1)	neg (1)
Diethylphthalate		pos (2)	neg (2)			
Diethylstilbesterol	pos (8)	pos (11)	pos (4)			
Dihexylphthalate	pos (1/2)	neg (1)	neg (2)			
Dihydrogenistein		pos (2)	pos (1)			
Dihydroglycitein		pos (2)	neg (1)			
Dihydrotestosterone	pos (1)	pos (3/4)				
Diisobutylphthalate		pos (2)	pos (2)			
Diisodecyl phthalate	neg (1)	neg (1)	neg (2)			
Diisononyl phthalate	neg (1)	pos (1)	pos (2)			
Dimethyl isophthalate		neg (1)	neg (1)			
Dimethyl sulfoxide	pos (2/3)	pos (1)	neg (1)			

Substance	Agonism Assays			Antagonism Assays		
	Mammalian Cell Reporter Gene	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**	**Mammalian Cell Reporter Gene**	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**
Di-*n*-octyl phthalate	neg (1)	neg (1)				
Ditridecyl phthalate		neg (1)	neg (2)			
Diuron		pos (1)	neg (1)			
Endosulfan	pos (3/4)	pos (3)	pos (2)			
α-Endosulfan	pos (2)	pos (1)	pos (2)			
β-Endosulfan	pos (2)	pos (1)	pos (2)			
Equol	pos (2)	pos (4)	pos (1)			
17α-Estradiol	pos (2)	pos (5)	pos (2)			
17β-Estradiol	pos (59)	pos (31)	pos (36)	pos (2/5)	pos (1/2)	pos (1/2)
17β-Estradiolbenzoate	pos (1)	pos (1)				
Estratriene-3,6α,17β-triol	pos (1)		pos (1)			
Estratriene-3,6β,17β-triol	pos (1)		pos (1)			
Estriol	pos (1)	pos (4)	pos (2)			
Estrone	pos (3)	pos (5)	pos (5)			
Ethanol	pos (1/2)	pos (2/4)	neg (4)			
17α-Ethinyl estradiol	pos (2)	pos (3)	pos (2)			

Substance	Agonism Assays			Antagonism Assays		
	Mammalian Cell Reporter Gene	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**	**Mammalian Cell Reporter Gene**	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**
4-Ethylphenol		neg (1)	neg (1)			
Fenarimol		pos (1)	pos (1)			
Flavone	pos (2/5)		neg (2)	pos (3)		pos (1)
Fluoranthene		neg (1)	neg (1)		neg (2)	neg (1)
Fluorene	pos (1/2)	neg (1)	neg (1)		neg (2)	neg (1)
Formononetin	pos (3)	pos (2/3)		neg (2)	pos (2)	
Genistein	pos (11)	pos (8)	pos (4/5)	pos (1/3)	neg (2)	pos (1/3)
Genistin		pos (1/4)	pos (1)			
Glycitein		pos (2/3)	neg (1)			
Glycitin		neg (2)	neg (1)			
Heptachlor	neg (1)		neg (2)			
2,2’,3,4,4’,5,5’-Heptachlorobiphenyl	neg (1)		neg (1)	pos (2)		pos (1)
2,2’,3’,4,4’,5,5’-Heptachloro-3-biphenylol	pos (1)		neg (1)	pos (1)		pos (1)
2,2’,3,4’,5,5’,6-Heptachloro-4-biphenylol	pos (1)		neg (1)	pos (1)		pos (1)
Hexachlorobenzene	neg (1)		neg (1)			

Substance	Agonism Assays			Antagonism Assays		
	Mammalian Cell Reporter Gene	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**	**Mammalian Cell Reporter Gene**	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**
2,2',3,4,4',5'-Hexachlorobiphenyl	neg (1)		neg (1)	pos (2)		pos (1)
2,2',4,4',5,5'-Hexachlorobiphenyl	neg (1)		neg (1)	pos (2)		pos (1)
2,2',3,3',4',5-Hexachloro-4-biphenylol	neg (1)		neg (1)	neg (1)		pos (1)
β-Hexachlorocyclohexane	neg (1)		pos (1)			
Hexestrol	pos (1)	pos (1)				
2-Hydroxyestradiol	pos (2)	pos (1)				
4-Hydroxytamoxifen	pos (3/8)	pos (1/4)		pos (10)	pos (5)	pos (1)
6-Hydroxytetralin	pos (1)		neg (1)			
ICI 164,384	neg (1)	pos (2/4)	neg (1)	pos (5)	pos (2)	pos (1)
ICI 182,780	pos (1/10)	pos (2)	neg (1)	pos (14/15)	neg (2)	pos (3)
Indeno(1,2,3-*cd*)pyrene	neg (1)	neg (1)	neg (1)		neg (2)	pos (1)
Kaempferide	pos (1/3)		neg (1)	pos (3)		pos (1)
Kepone	pos (4/6)	pos (1)	pos (2)	neg (2)		
Lindane	pos (1/2)	pos (1)	neg (1)			
Linuron	neg (1)		neg (1)			

Substance	Agonism Assays			Antagonism Assays		
	Mammalian Cell Reporter Gene	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**	**Mammalian Cell Reporter Gene**	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**
Luteolin				pos (1)		pos (1)
Methoxychlor	pos (11/12)	pos (8/9)	pos (2/3)	neg (2)	neg (1)	
Mirex	neg (2)		neg (1)			
Nafoxidine	neg (1)	pos (1/2)				
Naphthalene	neg (1)	neg (1)			neg (2)	
cis-Nonachlor	pos (1)	pos (1)				
trans-Nonachlor	pos (1)	pos (1)				
Nonylphenol	pos (1)	pos (6)				
n-Nonylphenol	pos (3)	pos (1)	pos (3)			
p-Nonylphenol	pos (4)	pos (13/14)	pos (4)			
Nordihydroguaiaretic acid	neg (1)	pos (1)				
19-Nortestosterone	pos (1)	neg (1)				
Octylphenol	pos (2)	pos (1)	pos (2)			
4-Octylphenol	pos (2)	pos (1)	pos (1)			
4-*t*-Octylphenol	pos (2/3)	pos (8/9)	pos (1/2)			
Parathion	neg (2)		neg (1)			

Substance	Agonism Assays			Antagonism Assays		
	Mammalian Cell Reporter Gene	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**	**Mammalian Cell Reporter Gene**	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**
2’,3,3’,4’,5-Pentachloro-4-biphenylol	neg (1)		neg (1)	pos (2)		pos (1)
Pentachlorophenol		pos (1)	neg (1)			
Permethrin	neg (1)		pos (1)			
Phenanthrene		neg (1)	neg (1)		neg (2)	neg (1)
Phenol		neg (1)	neg (1)			
Picloram		pos (1)	neg (1)			
Prochloraz		pos (1)	neg (1)			
Progesterone	neg (2)	pos (1/3)	neg (1)			
4-Propylphenol		pos (1)	neg (1)			
Pyrene	pos (1)		neg (1)			
Pyrethrins	neg (1)		neg (3)			
Quercetin	pos (2)		neg (1)	neg (2)		pos (1)
Raloxifene	neg (5)		neg (1)	pos (5)		pos (2)
Simazine	pos (1/2)		neg (1)			
β-Sitosterol	neg (2)	pos (1)	pos (1)			

Substance	Agonism Assays			Antagonism Assays		
	Mammalian Cell Reporter Gene	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**	**Mammalian Cell Reporter Gene**	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**
Sumithrin	neg (1)		pos (1)			
Tamoxifen	pos (5/7)	pos (1/3)	neg (2)	pos (7/8)	pos (1)	pos (2)
Testosterone	neg (2)	pos (5/9)				
Tetrabromobisphenol A	neg (1)	neg (1)	pos (1)			
2,2’,5,5’-Tetrachlorobiphenyl	neg (1)		neg (2)			neg (2)
3,3’,5,5’-Tetrachlorobiphenyl	pos (1)		pos (2)			neg (2)
2’,3’,4’,5’-Tetrachloro-4-biphenylol	pos (7/8)	pos (2)	pos (1)	pos (1/2)		neg (1)
3,3’,5,5’-Tetrachloro-4,4’-biphenylol	pos (1)	pos (1)				
2,3,7,8-Tetrachlorodibenzo-p-dioxin	pos (1)	pos (1)	neg (3)	pos (1)		pos (3)
Toxaphene	pos (2/3)	pos (2)	pos (3)			
Tralomethrin	pos (1)		pos (2)			
Triadimefon		pos (1)	pos (1)			
2’,4’,6’-Trichloro-4-biphenylol	pos (6/7)	pos (2)	pos (2)	pos (1)		neg (2)
Trifluralin		pos (1)	neg (1)			
α-Zearalanol	pos (2)	pos (3)	pos (2)			

Substance	Agonism Assays			Antagonism Assays		
	Mammalian Cell Reporter Gene	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**	**Mammalian Cell Reporter Gene**	**Yeast Reporter Gene**	**Mammalian Cell Proliferation**
β-Zearalanol		pos (3)	pos (1)			
Zearalanone		pos (2)	pos (1)			
α-Zearalenol	pos (1)	pos (3)	pos (1)			
β-Zearalenol	pos (3)	pos (2/3)	pos (1)			
Zearalenone	pos (9)	pos (3)	pos (3)	pos (2/3)		neg (1)
Zeranol	pos (1)		pos (2)			

* Numbers in parenthesis refer to the number of different assays in which the substance was tested; if the results were variable, the first number in parenthesis refers to the number of assays in which the substance was positive while the second number indicates the total number of tests conducted.

Abbreviations: *o,p'*-DDD = 2,4'-Dichlorodiphenyldichloroethane; *p,p'*-DDD = 2,2-Bis(4-chlorophenyl)-1,1-dichloroethane; *o,p'*-DDE = 1,1-Dichloro-2-(*o*-chlorophenyl)-2-(*p'*-chlorophenyl)ethylene; *p,p'*- DDE = 1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene; *o,p'*-DDT = 2-(*o*-Chlorophenyl)-2-(*p*-chlorophenyl)-1,1,1-trichloroethane; *p,p'*-DDT = 1,1,1-Trichloro-2,2-bis[*p*-chlorophenyl]ethane

Appendix F

Substances Tested in Different *In Vitro* ER TA Assays by the Same Investigator

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Substances Tested in Different In Vitro ER TA Assays by the Same Investigator

Bonefeld-Jørgensen et al. (2001)

Substance	Agonism		Antagonism		
	MCF-7 hER(E)+CAT(T)+β-gal(T)	MCF7 hER(E)+CP	MCF-7 hER(E)+CAT(T)+β-gal(T)	MCF7 hER(E)+CP	MDA-MB-231 hERα(T)+Luc(T)
2,2',4,4',5,5'-Hexachlorobiphenyl	neg	neg	pos	pos	pos
2,2',3,4,4',5'-Hexachlorobiphenyl	neg	neg	pos	pos	pos
2,2',3,4,4',5,5'-Heptachlorobiphenyl	neg	neg	pos	pos	pos
ICI 182,780	neg		pos		pos

Chen et al. (1997)

Substance	Agonism	
	Yeast(*S.cer.* 939) hER(S)+β-gal(S)	Yeast(*S.cer.* CYT10-5d) hER(S)+β-gal(S)
Dexamethasone	neg	neg
17β-Estradiol	pos	pos
p,p '-DDT	pos	pos
o,p '-DDD	pos	pos
p,p '-DDE	neg	neg
p,p '-DDA	neg	neg
o,p '-DDT	pos	pos
o,p '-DDE	pos	pos
ICI 164,384	pos	neg

Substances Tested in Different In Vitro ER TA Assays by the Same Investigator

Collins-Burow et al. (2000)

Substance	Agonism				Antagonism			
	HEK-293 hERα(T)+Luc(T)	HEK-293 hERα(T)+Luc(T)	MCF-7(M) hER(E) +Luc(T)+β-gal(T)	MCF-7(M) hER(E)+CP	HEK-293 hERα(T)+ Luc(T)	HEK-293 hERα(T)+ Luc(T)	MCF-7(M) hER(E) +Luc(T)+β-gal(T)	MCF-7(M) hER(E)+CP
17β-Estradiol	pos	pos	pos	pos	pos			
4-Hydroxytamoxifen			neg				pos	pos
Apigenin	pos	weak	pos	neg	pos	pos	pos	pos
Chrysin			pos				pos	pos
Flavone	neg	neg	neg	neg	pos	pos	pos	pos
Kaempferide	neg	neg	pos	neg	pos	pos	pos	pos
Luteolin			pos				pos	pos
Tamoxifen	pos	pos		neg	pos	pos	pos	pos

Connor et al. (1997)

Substance	Agonism		Antagonism	
	HeLa hERαdef (S)+Luc(S)	MCF-7 hER(T)+CAT(T)	HeLa hERαdef (S)+Luc(S)	MCF-7 hER(T)+CAT(T)
17β-Estradiol	pos	pos		neg
2',3,3',4',5'-Pentachloro-4-biphenylol	neg	neg	pos	pos
2,2',4',6'-Tetrachloro-4-biphenylol	pos	neg	pos	pos
2,2',3',4',6'-Pentachloro-4-biphenylol	pos	pos	pos	neg
2,2',3',5',6'-Pentachloro-4-biphenylol	neg	neg	pos	pos
2,2',3',4',5'-Pentachloro-4-biphenylol	neg	pos	pos	neg
2',3,4',6'-Tetrachloro-4-biphenylol	pos	neg	pos	pos
2',3,3',5',6'-Pentachloro-4-biphenylol	neg	neg	pos	pos
2',3,3',4',6'-Pentachloro-4-biphenylol	pos	neg	neg	pos

Substances Tested in Different In Vitro ER TA Assays by the Same Investigator

Fertuck et al. (2001b)

Substance	Agonism	
	MCF-7 hERαdef(T) +Luc(T)+β-gal(T)	MCF-7 mERαdef(T) +Luc(T)+β-gal(T)
17β-Estradiol	pos	pos
Benz[*a*]anthracene	weak	weak
Benzo[*c*]phenanthrene	neg	neg
Chrysene	neg	neg
Benzo[*a*]carbazole	weak	weak
Benzo[*b*]naphtho(2,1-d)thiophene	weak	weak
Benzo[*b*]fluorene	weak	weak
Benzo[*b*]naphtho(2,3-d)thiophene	weak	weak
2-Hydroxybenzo[*c*]phenanthrene	weak	weak
2-Hydroxychrysene	weak	pos
Benzo[*c*]carbazole	weak	weak
3-Hydroxybenzo[*b*]naphtho(2,3-*d*)thiophene	weak	weak
3-Hydroxy-benzo[*b*]phenanthro (2,3-*d*)thiophene	pos	pos
2-Hydroxy-5-methylchrysene	weak	pos
8-Hydroxy-5-methylchrysene	weak	weak

Fielden et al. (1997)

Substance	Agonism		Antagonism	
	MCF-7 hER(E)+CP	MCF-7 hER(T)+Luc(T)+β-gal(T)	MCF-7 hER(E)+CP	MCF-7 hER(T)+Luc(T)+b-gal(T)
17β-Estradiol	pos	pos		
2,2',4,4',6,6'-Hexachlorobiphenyl (PCB 155)	pos	neg	pos	pos
2,2',4,6,6'-Pentachlorobiphenyl (PCB 104)	pos	pos	neg	neg

Gaido et al. (1999)

Substance	Agonism				Antagonism			
	HepG2 hERα(T) +Luc(T)+β−gal(T)	HepG2 hERβ(T) +Luc(T)+β−gal(T)	HeLa hERα(T)+Luc(T)+β-gal(T)	HeLa hERβ(T)+Luc(T)+β-gal(T)	HepG2 rERα(T) +Luc(T)+β−gal(T)	HepG2 hERβ(T) +Luc(T)+β−gal(T)	HeLa hERα(T)+Luc(T)+β-gal(T)	HeLa hERβ(T)+Luc(T)+β-gal(T)
17β-Estradiol	pos	pos	pos	pos				
HPTE	pos	pos	pos	pos	neg	pos	pos	pos

Gaido et al. (1999) continued

Substance	Agonism	Antagonism
	HepG2 hERα(T) +Luc(T)+β−gal(T)	HepG2 hERα(T) +Luc(T)+β−gal(T)
17β-Estradiol	pos	
HPTE	pos	neg

Substances Tested in Different In Vitro ER TA Assays by the Same Investigator

Gaido et al. (2000)

Substance	Agonism		Antagonism	
	HepG2 hERα(T) +Luc(T)+β–gal(T)	HepG2 hERβ(T) +Luc(T)+β–gal(T)	HepG2 hERα(T) +Luc(T)+β–gal(T)	HepG2 hERβ(T) +Luc(T)+β–gal(T)
17β-Estradiol	pos	pos		
Methoxychlor	pos	pos	neg	neg
p,p' -DDE	pos	pos	neg	neg
Bisphenol A	pos	pos	neg	neg
Bis(4-hydroxyphenyl)ketone	pos	pos	neg	neg
Bis(4-hydroxyphenyl)methane	pos	pos	neg	neg
4,4'-Dimethoxybenzhydrol	weak	pos	neg	neg
HPTE	pos	pos	neg	pos
Bis(4-hydroxyphenyl)ethane	pos	pos	neg	neg
Monohydroxymethoxychlor	pos	pos	neg	pos
Dihydroxy-DDE	pos	pos	neg	pos
Dimethoxy-DDE	pos	pos	neg	neg
Monohyroxy-DDE	pos	pos	neg	pos
Trihydroxymethoxychlor	pos	pos	neg	neg
Trimethoxymethoxychlor	pos	pos	neg	neg

Harris et al. (1997)

Substance	Agonism		
	MCF-7 hER(E)+CP	Yeast(*S.cer.*) hER(S)+β-gal(S)	ZR-75 hER(E)+CP
17β-Estradiol	pos	pos	pos
Bis(2-ethylhexyl) phthalate (DEHP)	neg	neg	neg
Butyl benzyl phthalate (BBP)	pos	pos	pos
Dibutyl phthalate (DBP)	pos	pos	pos
Diethyl phthalate (DEP)	neg	pos	neg
Dihexyl phthalate (DHP)	neg	neg	neg
Diisobutyl phthalate (DIBP)	pos	pos	pos
Diisodecyl phthalate (DIDP)	neg	neg	neg
Diisononyl phthalate (DINP)	pos	weak	pos
Ditridecyl phthalate (DTDP)	neg	neg	neg
Ethanol	neg	neg	neg

Substances Tested in Different In Vitro ER TA Assays by the Same Investigator

Jobling et al. (1995)

Substance	Agonism	
	MCF-7 hER(E) +Luc(T)+β-gal(T)	ZR-75 hER(E)+CP
Di-*n* -butyl phthalate	pos	pos
Butyl benzyl phthalate	pos	pos
Bis(2-ethylhexyl)phthalate	weak	neg
Butylated hydroxytoluene	weak	pos
Butylated hydroxyanisole	pos	pos
Octylphenol	pos	pos
17β-Estradiol	pos	pos

Klotz et al. (1996)

Substance	Agonism	
	Yeast(*S.cer.BJ2407*) hER(S)+β-gal(S)	MCF-7 hER(E) +Luc(T)+β-gal(T)
17β-Estradiol	pos	pos
o,p '-DDD	pos	pos
p,p' -DDD	pos	pos
o,p '-DDT	pos	pos
cis -Nonachlor	pos	pos
Alachlor	pos	pos
Benomyl	neg	neg
trans -Nonachlor	pos	pos

Klotz et al. (1997)

Substance	Agonism		Antagonism	
	Ishikawa hER(T) +Luc(T)+β-gal(T)	MCF7 hER(E) +Luc(T)+β-gal(T)	Ishikawa hER(T) +Luc(T)+β-gal(T)	MCF7 hER(E) +Luc(T)+β-gal(T)
Parathion	neg	neg	pos	pos
Carbaryl	neg	neg	pos	pos
Baygon	neg	neg	pos	pos
Aldicarb	neg	neg	pos	pos
Methomyl	neg	neg	pos	pos
Bendiocarb	neg	neg	pos	pos
Oxamyl	neg	neg	pos	pos
17β-Estradiol	pos	pos	pos	pos

Substances Tested in Different In Vitro ER TA Assays by the Same Investigator

Kuiper et al. (1998)

Substance	Agonism		Antagonism	
	HEK-293 hERα(T) +Luc(T)+β-gal(T)	HEK-293 hERβ(T) +Luc(T)+β-gal(T)	HEK-293 hERα(T) +Luc(T)+β-gal(T)	HEK-293 hERβ(T) +Luc(T)+β-gal(T)
p,p '-DDT	pos	pos		
Diethylstilbestrol	pos	pos		
Phloretin	pos	pos	neg	neg
Methoxychlor	pos	pos		
Bisphenol A	pos	pos		
4,4'-Biphenyldiol	pos	pos		
α,β-Endosulfan	pos	neg		
Quercetin	pos	pos	neg	neg
4-*tert* -Octylphenol	pos	pos		
Kepone	pos	neg	neg	neg
Genistein	pos	pos	neg	neg
Coumestrol	pos	pos	neg	neg
Chrysin	neg	pos	neg	neg
Naringenin	pos	pos	neg	neg
Formononetin	pos	pos	neg	neg
Daidzein	pos	pos	neg	neg
Biochanin A	pos	pos	neg	neg
Kaempferol	pos	pos	neg	neg
Apigenin	pos	pos	neg	neg
Flavone	pos	pos		
o,p '-DDT	pos	pos		
4-Octylphenol	pos	pos		
Tamoxifen	pos	pos	pos	neg
2',4',6'-Trichloro-4-biphenylol	pos	pos		
Zearalenone	pos	pos	neg	pos
Nonylphenol	pos	pos		
Ipriflavone	pos	pos	neg	neg
2',3',4',5'-Tetrachloro-4-biphenylol	pos	pos		
ICI 182,780	neg	pos		neg
2,3,3',4',5'-Pentachloro-4-biphenylol	neg	neg	neg	neg
2,2',4',6'-Tetrachloro-4-biphenylol	pos	pos	neg	neg
17β-Estradiol	pos	pos		

Substances Tested in Different In Vitro ER TA Assays by the Same Investigator

Le Guevel & Pakdel (2001)

Substance	Agonism		
	Yeast(*S.cer.BJ-ECZ*) hER(S)+β-gal(S)	Yeast(*S.cer.BJ-ECZ*) rtER(S)+β-gal(S)	Ishikawa hER(E)+CP
17β-Estradiol	pos	pos	pos
Estrone	pos	pos	pos
Diethylstilbestrol	pos	pos	pos
17α-Ethinyl estradiol	pos	pos	pos
17α-Estradiol	pos	pos	pos
Zearalanone	pos	pos	pos
Zearalenone	pos	pos	pos
α-Zearalanol	pos	pos	pos
α-Zearalenol	pos	pos	pos
β-Zearalanol	pos	pos	pos
β-Zearalenol	pos	neg	pos

Makela et al. (1994)

Substance	Agonism			
	HeLa mER(T)+CAT(T)	HeLa mER(T)+CAT(T)*	MCF-7 hER(E)+CP	T47D hER(E)+CP
Estrone			pos	pos
Coumestrol	pos	pos	pos	pos
Biochanin A		pos	pos	
Zearalenone	pos	pos	pos	
Zeranol		pos	pos	
Genistein		pos	*pos*	*pos*
17β-Estradiol	pos	pos	pos	

Matthews et al. (2001)

Substance	Agonism	
	MCF7 hERαdef(T) +Luc(T)+β-gal(T)	MCF7 hERβdef(T) +Luc(T)+β-gal(T)
Bisphenol A	pos	pos
17β-Estradiol	pos	pos
Bisphenol A glucuronide	neg	neg

Meerts et al. (2001)

Substance	Agonism	
	T47D hER(E)+Luc(S)	HEK293+ hERα(S)+Luc(S)
17β-Estradiol	pos	pos/pos
4-(2,4,6-Tribromophenoxy)phenol	neg	pos/pos
4-Phenoxyphenol	pos	pos/pos
Polybrominated diphenyl ether 30	pos	pos/pos
Polybrominated diphenyl ether 100	pos	pos/neg

Substances Tested in Different In Vitro ER TA Assays by the Same Investigator

Meyers et al. (1999)

Substance	Agonism		Antagonism	
	HEC-1 hERβ(T) +CAT(T)+ β-gal(T)	HEC-1 hERα(T) +CAT(T)+ β-gal(T)	HEC-1 hERβ(T) +CAT(T)+ β-gal(T)	HEC-1 hERα(T) +CAT(T)+ β-gal(T)
17β-Estradiol	pos	pos		
(5R,11R)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol	neg	pos	pos	neg
(5S,11S)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol	pos	pos	pos	neg
(5R,11R)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol	neg	pos	pos	neg
(5S,11S)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol	pos	pos	neg	neg
5,11-*trans* -Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol	pos	pos	neg	neg
(5R,11R)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8 diol	neg	pos	pos	neg
(5S,11S)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8 diol	neg	pos	pos	neg
5,11-*trans* -Dipropyl-5,6,11,12-tetrahydrochrysene-2,8 diol	pos	pos	neg	neg

Moore et al. (1997)

Substance	Agonism		Antagonism	
	HeLa hER(S) +Luc(S)	MCF-7 hER(E)+CP	HeLa hER(S) +Luc(S)	MCF-7 hER(E)+CP
17β-Estradiol	pos	pos		
2,2',3,3',4',5-Hexachloro-4-biphenylol	neg	neg	neg	pos
2,2',3,4',5,5',6-Heptachloro-4-biphenylol	pos	neg	pos	pos
2,2',3',4,4',5,5'-Heptachloro-3-biphenylol	pos	neg	pos	pos
2',3,3',4',5-Pentachloro-4-biphenylol	neg	neg	pos	pos

Substances Tested in Different In Vitro ER TA Assays by the Same Investigator

Morito et al. (2001a)

Substance	Agonism		
	Yeast(S.cer.190) hERα(S)+β-gal(S)	Yeast(S.cer.190) hERβ(S)+β-gal(S)	MCF-7 hER(E)+CP
17β-Estradiol	pos	pos	pos
Diethylstilbestrol	pos	pos	pos
Bisphenol A	pos	pos	pos
Genistein	pos	pos	pos
Daidzein	pos	pos	pos
Genistin	neg	pos	pos
Equol	pos	pos	pos
Daidzin	pos	neg	pos
Tamoxifen	neg	neg	
Dihydrogenistein	pos	pos	pos
Nonylphenol	pos	pos	pos
Dihydroglycitein	pos	pos	neg
Glycitein	pos	pos	neg
Glycitin	neg	neg	neg

Morito et al. (2001b)

Substance	Agonism		Antagonism	
	Yeast(S.cer.190) hERα(S)+β-gal(S)	Yeast(S.cer.190) hERβ(S)+β-gal(S)	Yeast(S.cer.190) hERα(S)+β-gal(S)	Yeast(S.cer.190) hERβ(S)+β-gal(S)
17β-Estradiol	pos	pos		
Genistein	pos	pos	neg	neg
Coumestrol	pos	pos	neg	neg
Formononetin	neg	pos	pos	*pos*
Biochanin A	neg	neg	pos	pos
Genistin	neg	neg	neg	neg
Tectorigenin	pos	pos	neg	pos
Tectoridin	neg	pos	neg	neg
Sissotorin	neg	pos	neg	neg
4-Hydroxytamoxifen	neg	neg	pos	pos
5-OMe-Genistein	neg	pos	pos	pos
7-OMe-Genistein	neg	pos	pos	pos
Irisolidone	neg	pos	pos	pos
Irisolidone-7-O-beta-D-glucoside	neg	neg	neg	neg

Substances Tested in Different In Vitro ER TA Assays by the Same Investigator

Otsuka Pharmaceutical (2001)

Substance	Agonism		
	MCF-7 hER(E)+CP	CHO-K1 hERα(T)+Luc(T)†	CHO-K1 hERα(S)+Luc(S)†
17β-Estradiol	pos	pos	
2,2-Bis(p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE)		pos	pos
4-Nonylphenol		pos	pos
Acrinathrin	neg	neg	
Bisphenol A		pos	pos
Bisphenol B		pos	pos
Cycloprothrin	neg	pos	
Cyfluthrin	pos	pos	
Cyhalothrin	pos	pos	
Cypermethrin	pos	neg	
Deltamethrin	pos	pos	
Diethylstilbestrol		pos	pos
Equol		pos	pos
17α-Ethinyl estradiol		pos	pos
Levonorogestrel		pos	neg
Methyltestosterone		pos	pos
p -Cumylphenol		pos	pos
Pyrethrins	neg	neg	
Tralomethrin	pos	pos	
Zearanol		pos	pos

Ramamoorthy et al. (1997a)

Substance	Agonism		
	MCF-7 hER(E)+CP	MCF-7 hER(T)+CAT(T)	Yeast(*S.cer.* BJ2168) mER(S)+β-gal(S)
17β-Estradiol	pos	pos	pos
Dieldrin	neg	pos	pos
Toxaphene	pos	pos	pos

Ramamoorthy et al. (1997b)

Substance	Agonism				
	HepG2 hER(T) +Luc(T)+β-gal(T)	MDA-MB-231 +hER(T)+Luc(T)	MCF-7 +hER(T)+CAT(T)	Yeast(*S.cer.* BJ3503) hER(S)+β-gal(S)	MCF-7 hER(T)+Luc(T)
17β-Estradiol	pos	pos	pos	pos	pos
2',4',6'-Trichloro-4-biphenylol	pos	pos	pos	pos	pos
2',3',4',5'-Tetrachloro-4-biphenylol	pos	pos	pos	pos	pos

Substances Tested in Different In Vitro ER TA Assays by the Same Investigator

Seinen et al. (1999)

Substance	Agonism			
	HEK293 hERα(S)+Luc(T)	HEK-293 hERα(T)+Luc(T)	HEK-293 hERβ(S)+Luc(T)	HEK-293 hERβ(T)+Luc(T)
17β-Estradiol	pos	pos	pos	pos
6-Acetyl-1,1,2,4,4,7-hexamethyltetraline	neg	pos	neg	neg
1,2,4,6,7,8-Hexahydro-4,6,6,7,8-hexamethylcyclopenta-y-2-benzopyrene	neg	pos	neg	neg

Sun et al. (1999)

Substance	Agonism				Antagonism	
	HEC-1 hERα(T) +CAT(T)+β-gal(T)	HEC-1 hERα(T) +Luc(T)+β-gal(T)	HEC-1 hERβ(T) +CAT(T)+β-gal(T)	HEC-1 hERβ(T) +Luc(T)+β-gal(T)	HEC-1 hERβ(T) +CAT(T)+β-gal(T)	HEC-1 hERβ(T) +Luc(T)+β-gal(T)
17β-Estradiol	pos	pos	pos	pos		
cis,cis -Diethyldihydroxy tetrahydrochrysene	pos	pos	neg	neg	pos	pos
R,R-cis,cis -Diethyldihydroxy tetrahydrochrysene	pos		neg		pos	
S,S-cis,cis -Diethyldihydroxy tetrahydrochrysene	pos		pos		pos	

Tremblay et al. (1998)

Substance	Agonism				Antagonism			
	COS-1 mERα(T)+Luctk(T)	COS-1 mERα(T)+Lucb(T)	COS-1 mERβ(T)+Luctk(T)	COS-1 mERβ(T)+Lucb(T)	COS-1 mERα(T)+Luctk(T)	COS-1 mERα(T)+Lucb(T)	COS-1 mERβ(T)+Luctk(T)	COS-1 mERβ(T)+Lucb(T)
17β-Estradiol	pos	pos	pos	pos				
EM-652	neg	neg	neg	neg	pos	pos	pos	pos
4-Hydroxytamoxifen	neg	pos	neg	neg	pos	pos	pos	pos
Droloxifene	neg	pos	neg	neg	pos	pos	pos	pos
Raloxifene	neg	neg	neg	neg	pos	pos	pos	pos
Hydroxytoremifene	neg	pos	neg	neg	pos	pos	pos	pos
ICI 182,780	neg	neg	neg	neg	pos	pos	pos	pos

Vinggaard et al. (1999)

Substance	Agonism	
	MCF-7(E3) hER(E)+CP	Yeast(*S.cer.*) hERα(S)+β gal(S)
17β-Estradiol	pos	pos
Dicofol	neg	pos
Fenarimol	pos	pos

Substances Tested in Different In Vitro ER TA Assays by the Same Investigator

Zacharewski et al. (1998)

Substance	Agonism	
	HeLa hER(S)+Luc(S)	MCF-7 hER(T) +Luc(T)+β-gal(T)
17β-Estradiol	pos	pos
Dibutyl phthalate	neg	pos
Dihexylphthalate	neg	
Butyl benzyl phthalate	pos	pos
Diethylhexyl phthalate	neg	neg
Di-*n* -octyl phthalate	neg	neg
Diisodecyl phthalate	neg	neg
Diisononyl phthalate	neg	neg
Diisoheptyl phthalate	neg	neg