

Comparative Analysis of Substances Recommended for Validation of *In Vitro* ER Binding and *In Vitro* ER Transcriptional Activation (TA) Assays

In both the *in vitro* ER binding and the *in vitro* ER TA assay BRDs, a list of recommended substances for future validation efforts were proposed (**Section 12**). Each list included substances covering a range of activities (from highly active to non-active) in the respective assays. Ideally, to allow for a direct comparison of performance and reliability between binding and TA assays, the same set of substances should be tested in both types of assays. To help in the selection of a coordinated list, the binding and TA responses for substances included in each list are provided in **Table 1**. The data are organized alphabetically by substance.

For each substance, the nature of the response (positive or negative) and the corresponding number of assays in which it was tested (shown in parenthesis) are provided. In cases where discordant data were obtained across assays, two values are provided in parenthesis. The first value indicates the number of positive assays, while the second value indicates the number of assays in which the substance was tested. In determining the call for each assay, a positive response was assigned if at least one positive test was reported. This approach was used to be consistent with the approach used in the BRD and was based on the observation that negative test data (in situations where discordant test results within an assay were reported) appeared to be more often the result of inadequate dose selection rather than a lack of intrinsic assay sensitivity. The number of plus signs (for positive substances) indicates the relative potency of the substance in the assay considered. In the ER binding assay, three pluses indicate that the median RBA value across assays was greater than 0.1, two pluses indicate the median RBA value ranged between 0.001 and 0.1, and one plus indicates that the RBA value was less than 0.001. Since there was little quantitative data for the TA assays, it was not possible in all cases to assign an indication of potency. In the absence of EC₅₀ data (for agonism assays), a single plus was used to identify a positive response. In assays where a quantitative measure of activity was available, an additional plus was assigned if the median EC₅₀ value was greater than 0.001 μ M and two pluses if the EC₅₀ value was lower than 0.001 μ M. Due to the lack of IC₅₀ data for antagonism assays, a single plus was used to identify all positive substances.

Substances that were suggested for validation in each of the assays are indicated with an asterisk. Currently, 18 substances are proposed in common for testing in *in vitro* ER binding and TA assays.

Table 1 Comparative Activity of Recommended Validation Substances in *In Vitro* ER Binding and *In Vitro* ER TA Assays

Substance	CASRN	ER Binding Assays [†]	ER TA Agonism Assays ^{††}	ER TA Antagonism Assays
4-Androstenedione	63-25-2	+ (1/3)*	- (3)	not tested
Apigenin	520-36-5	+ (3)	+ (5/5)	+ (4/6)*
Atrazine	1912-24-9	+ (1/6)*	+ (6)*	- (3)*
Bendiocarb	22781-23-3	not tested	- (2)	+ (1)*
Bisphenol A	80-50-7	+ (14)*	++ (17)*	- (2)*
Bisphenol C	14868-03-2	++ (3)*	Not tested	not tested
4- <i>tert</i> -Butylphenol	98-54-4	+ (1)*	+++ (2)	not tested
4-Chloro-4'-biphenylol	28034-99-3	+ (2)*	+ (2)	- (1)
Chlordane	57-74-9	- (1)	+ (2/3)*	not tested
Chlordecone	143-50-0	+ (9/10)*	+ (6/8)	+ (2/2)
Coumestrol	479-13-0	++ (11)*	++ (6)*	- (2)*
<i>o,p'</i> -DDT	789-02-6	+ (10/12)*	++ (6/7)*	+ (1)
<i>p,p'</i> -DDT	50-29-3	+ (2/9)*	++ (2)*	not tested
Daidzein	486-66-8	+ (4)	++ (3)*	- (2)
Diben[<i>a,h</i>]anthracene	53-70-3	- (1)	+ (1/4)	+ (3)*
Dicofol	115-32-2	not tested	- (2)*	not tested
Diethylhexyl phthalate	117-81-7	- (1)*	+ (2/8)	- (2)
5 -Dihydrotestosterone	521-18-6	+ (9)*	+ (4/5)	not tested
Diethylstilbestrol	56-53-1	++ (14)*	+++ (10)*	not tested
Drolxifene	82413-20-5	+++ (3)	+ (1/2)	+ (4)*
Equol	531-95-3	+ (3)	++ (4)*	not tested
17 -Estradiol	57-91-0	++ (7)	+++ (6)*	not tested
17 -Estradiol	50-28-2	+++ (13)	+++ (56)*	+ (3/8)
Estriol	50-27-1	++ (11)*	++ (3)*	not tested
Estrone	53-16-7	++ (13)*	++ (6)*	not tested
17 - Ethinyl estradiol	57-63-6	+++ (7)	+++ (5)*	not tested
Flavone	525-82-6	- (3)	+ (2/6)	+ (3)*
Fluoranthene	206-44-0	not tested	- (2)*	- (2)*
Formononetin	485-72-3	+ (3)	+ (4/5)	+ (1/3)*
Genistein	446-72-0	+ (11/14)*	++ (9)*	+ (2/6)
Heptachlor	76-44-8	- (1)	- (2)*	not tested
4-Hydroxytamoxifen	68047-06-3	++ (14)*	+ (4/9)	+ (15)*
Hydroxytoremifene	110503-62-3	Not tested	+ (1/4)	+ (4)*
ICI 164,384	98007-99-9	+++ (9)	+ (3/6)	+ (8)*
ICI 182,780	129453-61-8	+++ (4)	+ (3/11)	+ (13/16)*
Kaempferide	491-54-3	not tested	+ (1/3)	+ (3)*
Kaempferol	520-18-3	+ (3)*	++ (2)	- (3)

Substance	CASRN	ER Binding Assays [†]	ER TA Agonism Assays ^{††}	ER TA Antagonism Assays
Levonorgestrel	797-63-7	not tested	+ (2)*	not tested
Mestranol	72-33-3	++ (2)*	+ (1)	not tested
Methoxychlor	72-43-5	+ (6/13)*	+ (6/7)*	- (2)
Methyl testosterone	58-18-4	not tested	++ (2)*	not tested
Mirex	2385-85-5	- (1)	- (2)*	not tested
Morin	480-16-0	+ (1)*	Not tested	not tested
Nafoxidine	1845-11-0	+ (5/6)*	+ (1/3)	not tested
Naringenin	480-41-1	+ (6/8)*	++ (1)*	+ (1/2)
Norethynodrel	68-23-5	+ (3)*	Not tested	not tested
19-Nortestosterone	434-22-0	+ (3)	++ (1)*	not tested
4-Nonylphenol	104-40-5	+ (5)	++ (6)*	1
4-Octylphenol	1806-26-4	+ (4/5)*	+ (6)	+ (1)*
4- <i>tert</i> -Octylphenol	140-66-9	+ (9)*	++ (4)*	not tested
Phloretin	60-82-2	+ (4)*	++ (4)*	+ (2/4)*
Progesterone	57-83-0	+ (1/2)*	+ (1/6)*	- (1)
Raloxifene	84449-90-1	+ (3)	- (6)	+ (7)*
Simazine	122-34-9	- (6)*	+ (1/4)	- (2)
-Sitosterol	83-46-5	- (8)*	+ (2/4)	not tested
Tamoxifen	10540-29-1	++ (14)*	+ (4/10)	+ (7/8)*
2',4',6',-Trichloro-4-biphenylyol	14962-28-8	++ (4)*	+ (6)	+ (1/2)
-Zearalanol	26538-44-3	not tested	+++ (4)*	+ (1)*
-Zearalenol	71030-11-0	+++ (6)	++ (5)*	+ (1)
Zearalenone	17924-92-4	++ (9)*	++ (12)*	+ (2/4)*

A single number in parenthesis indicates the number of assays in which the substance was tested. Two numbers in parenthesis indicate that the outcomes in the assays for that substance were discordant; the first number indicates the number of positive responses and the second, the number of assays in which the substance was tested.

- indicates that the substance was negative in the assay.

+ indicates that the substance was positive in the assay.

[†]+++ indicates an RBA value greater than 0.1; ++ indicates an RBA value between 0.1 and 0.0001; + indicates an RBA value below 0.0001.

^{††}+++ indicates an EC₅₀ value that is smaller than 0.001; ++ indicates an EC₅₀ value that is higher than 0.001; + indicates a positive response but where an EC₅₀ value was not reported.

* Substances suggested for validation in the designated assay.

For most substances tested there was general concordance between the response in the binding and the TA assays. However, two substances (4-androstendione and raloxifene) were positive for binding but negative in the TA assay for agonism. Raloxifene was positive also in the TA assay for antagonism. Five compounds (chlordan, dibenz[*a,h*]anthracene, diethylhexyl phthalate, flavone, and simazine) were negative in binding assays but positive in TA assays for agonism. Most of these substances had only been tested in a few assays; thus, this observation may not be significant.

