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 (intralaboratory reproducibility) or in different laboratories (interlaboratory 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**.