

Appendix D: Agonist Plate-to-Plate Data Summary

Compounds Tested by XDS's LUMI CELL™ ER Recombinant Assay for Plate-to-Plate Variability

N/A - Not Applicable ND - Not Determined

P - Positive PP- Presumed Positive

PN - Presumed Negative X - No Data Available

? - Data not clear

Agonist Plate-to-Plate Data

<u>ICCVAM Recommended Compounds for Validation of ER TA Assays</u>	CAS RN	ICCVAM Historical Data				XDS's LUMI CELL™ ER Data for Validation of <i>In Vitro</i> ER TA Assay						
		RAB Range	MCRG Studies			Estradiol Positive at 10 µg/ml	Estradiol Positive at 1 µg/ml	Cell Viability	<u>EC 50 mmol/ml ± Standard error</u>		Relative Induction to Estradiol	Plate-to-Plate Coefficient of Variation
			<u>ER</u>	<u>Agonist</u>	<u>Antagonist</u>				<u>EC 50 mmol/ml ± Standard error</u>			
<u>ER POSITIVE:</u>			<u>Agonist</u>	<u>Antagonist</u>	<u>Agonist</u>	<u>Antagonist</u>						
Apigenin	520-36-5	P	+	+	-	-	Cell Toxicity	+	Viable	5.30E-06 ± 1.15E-06	3.75E-06	57%
Bisphenol A	80-05-7	P	+	-	-	+		+	Viable	1.07E-06 ± 1.84E-07	1.86E-05	34%
Butylbenzyl phthalate	85-68-7	PP	+	-	-	-		+	Viable	1.94E-06 ± 8.42E-07	1.03E-05	75%
Coumesterol	479-13-0	P	+	+	-	-		+	Viable	4.30E-08 ± 4.01E-10	4.63E-04	76%
Daidzein	486-66-8	P	+	-	-	-		+	Viable	2.60E-06 ± 3.58E-07	7.65E-06	24%
Dexamethasone	50-02-2	PN	+	-	+	-		+	Viable	1.05E-05 ± 5.76E-06	1.89E-06	95%
p,p' -DDE	72-55-9	P	+	-	+	+		+	Viable	6.21E-06 ± 2.58E-06	3.20E-06	72%
p,p'-DDT	50-29-3	P	+	+	-	+		+	Viable	3.51E-06 ± 3.78E-07	5.68E-06	22%
Dibenzo(a,h)anthracene	53-70-3	PP	+	+	+	-	Insoluble	+	Viable	N/A	N/A	N/A
Di-n -butyl phthalate	84-74-2	PP	+	-	-	-		+	Viable	9.74E-06 ± 5.62E-06	2.04E-06	16%
Diethylstilbestrol	56-53-1	P	+	-	-	+		+	Viable	3.11E-11 ± 5.87E-12	6.39E-01	38%
17α-Estradiol	57-91-0	P	+	-	-	-		+	Viable	3.16E-09 ± 7.54E-10	6.31E-03	41%
17β-Estradiol	50-28-2	P	+	-	+	+		+	Viable	1.99E-11 ± 6.93E-13	1.00E+00	45%
17alpha-Ethynodiol Estradiol	57-63-6	P	+	-	-	-		+	Viable	1.61E-11 ± 6.63E-12	1.24E+00	71%
Estrone	53-16-7	P	+	-	+	-		+	Viable	6.10E-10 ± 1.89E-10	3.26E-02	69%
Ethyl paraben	120-47-8	P	+	-	-	-		+	Viable	1.26E-05 ± 7.05E-06	1.58E-06	97%
Fenarimol	60168-88-9	PP	+	-	-	+	Cell Toxicity	+	Viable	8.15E-06 ± 1.26E-06	2.44E-06	31%
Flavone	525-82-6	PN	+	+	-	-	Cell Toxicity	+	Viable	N/A	N/A	N/A
Genistein	446-72-0	P	+	+	-	-		+	Viable	7.90E-07 ± 2.52E-07	2.52E-05	55%

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			ER	Agonist	Antagonist	AR							
ER POSITIVE (cont.):													
Kaempferol	520-18-3	P	+	-	-	-	+	+	Viable	2.30E-06 ± 2.03E-07	8.64E-06	15%	
Kepone	143-50-0	P	+	-	-	+	+	+	Viable	2.00E-06 ± 3.97E-07	9.96E-06	34%	
Methoxychlor	72-43-5	PP	+	-	-	+	+	+	Viable	3.53E-06 ± 6.10E-07	5.63E-06	30%	
n-Nonylphenol	104-40-5	P	+	?	-	?	+	+	Viable	2.36E-07 ± 6.03E-08	8.43E-05	44%	
Norethynodrel	68-23-5	P	+	-	-	-	+	+	Viable	1.81E-04 ± 1.91E-05	1.10E-07	18%	
4-tert-Octylphenol	140-66-9	P	+	-	-	+	+	+	Viable	3.34E-07 ± 2.70E-08	5.97E-05	21%	
Tamoxifen	10540-29-1	P	+	+	-	-	-	-	Viable	N/A	N/A	N/A	
2,4,5-Trichlorophenoxyacetic acid	93-76-5	PN	+	-	-	-	+	+	Viable	1.30E-05 ± 2.60E-06	1.53E-06	35%	
Zearalenone	17924-92-4	P	+	+	-	-	+	+	Viable	9.80E-10 ± 4.82E-11	2.03E-02	7%	
ER NEGATIVE:													
Actinomycin D	50-76-0	PP	-	-	-	-	-	-	ND	Non-Active	N/A	N/A	
Ammonium perchlorate	7790-98-9	PP	-	-	-	-	-	-	ND	Non-Active	N/A	N/A	
4-Androstene	63-05-8	PP	-	-	+	-	+	+	Viable	3.49E-05 ± 1.24E-05	5.70E-07	61%	
Atrazine	1912-24-9	PP	-	-	-	-	+	+	Viable	9.43E-05 ± 4.697E-05	2.11E-07	86%	
2-sec-Butylphenol	89-72-5	PP	-	-	-	-	-	+	ND	5.04E-05 ± 1.33.E-05	3.95E-07	37%	
Corticosterone	50-22-6	PN	-	-	-	-	Cell Toxicity		+	Viable	4.41E-06 ± 6.30E-07	4.52E-06	25%
Cycloheximide	66-81-9	PP	-	-	-	-	-	-	ND	Non-Active	N/A	N/A	
Cyproterone acetate	427-51-0	PP	-	-	+	+	-	-	ND	Non-Active	N/A	N/A	
Diethylhexyl phthalate	117-81-7	PN	-	-	-	-	-	-	ND	Non-Active	N/A	N/A	
Flutamide	13311-84-7	PP	-	-	-	+	-	-	ND	Non-Active	N/A	N/A	
Haloperidol	52-86-8	PP	-	-	-	-	-	-	ND	Non-Active	N/A	N/A	
Ketoconazole	65277-42-1	PP	-	-	+	-	-	-	ND	Non-Active	N/A	N/A	
Linuron	330-55-2	PP	-	-	+	+	+	-	ND	1.45E-05 ± 6.25E-06	1.37E-06	74%	
Medroxyprogesterone Acetate	71-58-9	PP	-	-	+	-	Cell Toxicity		+	Viable	5.36E-05 ± 1.28E-05	3.71E-07	54%
Morin	480-16-0	PP	-	-	-	-	+	+	Viable	2.86E-05 ± 1.00E-06	6.96E-07	6%	
Nilutamide	63612-50-0	PP	-	-	+	+	-	-	ND	Non-Active	N/A	N/A	

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			ER		AR							
ER NEGATIVE (cont.):			Agonist	Antagonist	Agonist	Antagonist						
Phenolphthlin	81-90-3	PP	-	-	-	-	+	-	Viable	1.48E-04 ± 2.17E-05	1.34E-07	25%
Pimozide	2062-78-4	PP	-	-	-	-	-	-	ND	Non-Active	N/A	N/A
Procymidone	32809-16-8	PP	-	-	-	+	-	-	ND	Non-Active	N/A	N/A
Progesterone	57-83-0	PP	-	-	+	+	-	-	ND	Non-Active	N/A	N/A
Propylthiouracil	51-52-5	PP	-	-	-	-	-	-	ND	Non-Active	N/A	N/A
Reserpine	50-55-5	PP	-	-	-	-	-	-	ND	Non-Active	N/A	N/A
Spironolactone	52-01-7	PP	-	-	+	+	+	-	Viable	N/A	N/A	N/A
12-O -Tetradecanoylphorbol-13-acetate	16561-29-8	PP	-	-	-	-	-	-	ND	Non-Active	N/A	N/A
L-Thyroxine	51-48-9	PP	-	-	-	-	+	+	ND	3.69E-01 ± N/A	5.39E-11	N/A
Vinclozolin	50471-44-8	PP	-	-	-	+	-	-	ND	Non-Active	N/A	N/A
Environmental Contaminants Not on ICCVAM List for Validation												
POSITIVE:												
Acenaphthylene	208-96-8	X	X	X	X	X	ND	+	Viable	N/A	N/A	N/A
5b-Androstan-3A-OL-17-One	53-42-9	X	X	X	X	X	+	+	Viable	N/A	N/A	N/A
Arochlor 1016	12674-11-2	X	X	X	X	X	ND	+	Viable	N/A	N/A	N/A
Arochlor 1221	11104-28-2	X	X	X	X	X	ND	+	Viable	N/A	N/A	N/A
Arochlor 1232	11141-16-5	X	X	X	X	X	ND	+	Viable	N/A	N/A	N/A
Arochlor 1242	53469-21-9	X	X	X	X	X	ND	+	Viable	N/A	N/A	N/A
Arochlor 1248	12672-29-6	X	X	X	X	X	ND	+	Viable	N/A	N/A	N/A
Benzo(a)anthracene	56-55-3	X	X	X	X	X	+	+	Viable	5.08E-06 ± 4.51E-07	3.92E-06	13%
Benzo(a)pyrene	50-32-8	X	X	X	X	X	+	+	Viable	3.65E-06 ± 6.93E-07	5.45E-06	38%
Benzo(k)fluoranthene	207-08-9	X	X	X	X	X	+	+	Viable	4.33E-06 ± 6.64E-07	4.60E-06	41%
Biochanin A	491-80-5	X	X	X	X	X	+	+	Viable	7.76E-07 ± N/A	2.56E-05	N/A
α-Chlordane	57-74-9	X	X	X	X	X	+	+	Viable	8.73E-07 ± 2.01E-07	2.28E-05	40%
? -Chlorodane	12789-03-6	X	X	X	X	X	+	+	Viable	4.86E-06 ± 1.67E-06	4.09E-06	69%
Chrysene	218-01-9	X	X	X	X	X	+	+	Viable	7.15E-06 ± 6.24E-07	2.78E-06	17%

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			ER		AR							
POSITIVE (cont.):	Agonist	Antagonist	Agonist	Antagonist								
p-Cresol	106-44-5	X	X	X	X	X	ND	+	Viable	N/A	N/A	N/A
p-Cymene	99-87-6	X	X	X	X	X	+	-	Viable	Non-Active ± N/A	N/A	N/A
DDD	72-54-8	X	X	X	X	X	+	+	Viable	1.97E-06 ± 5.10E-07	1.01E-05	45%
Dieldrin	60-57-1	X	X	X	X	X	+	+	Viable	8.41E-06 ± 8.51E-07	2.37E-06	29%
α-Endosulfan	959-98-8	X	X	X	X	X	+	+	Viable	4.42E-06 ± 1.06E-06	4.50E-06	54%
β-Endosulfan	33213-65-9	X	X	X	X	X	+	+	Viable	3.34E-06 ± 1.93E-06	5.96E-06	35%
Endrin	72-20-8	X	X	X	X	X	+	+	Viable	1.19E-05 ± 4.23E-06	1.67E-06	50%
Fluorene	86-73-7	X	X	X	X	X	+	+	Viable	1.31E-04 ± 3.57E-05	1.52E-07	61%
Indeno(123,cd)pyrene	193-39-5	X	X	X	X	X	ND	+	Viable	7.04E-06 ± N/A	2.83E-06	N/A
Isodrin	465-73-6	X	X	X	X	X	+	+	Viable	1.14E-05 ± 2.74E-06	1.74E-06	42%
Lindane	58-89-9	X	X	X	X	X	+	+	Viable	2.07E-05 ± 8.45E-06	9.61E-07	43%
Naringenin	480-41-1	X	X	X	X	X	+	+	Viable	5.34E-06 ± 5.03E-07	3.73E-06	13%
2-Phenylindole	948-65-2	X	X	X	X	X	+	+	Viable	4.16E-06 ± 3.02E-06	4.78E-06	126%
4-Phenyl Toluene	644-08-6	X	X	X	X	X	+	+	Viable	4.46E-05 ± 4.01E-06	4.46E-07	20%
Pyrene	129-00-0	X	X	X	X	X	+	+	Viable	1.24E-05 ± 3.83E-06	1.60E-06	62%
2,4,5-Trichlorophenol	95-95-4	X	X	X	X	X	+	+	Viable	2.28E-05 ± 5.10E-06	8.74E-07	39%
α-Zearalenol	36455-72-8	X	X	X	X	X	+	+	Viable	2.77E-11 ± 4.25E-13	7.17E-01	3%
NEGATIVE:												
Arochlor 1254	11097-69-1	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A
Arochlor 1260	11096-82-5	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A
Carbazole	86-74-8	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A
Creosote	8001-58-9	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A
o-Cresol	95-48-7	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A
Cumene	98-82-8	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A
1,4 Dioxane	123-91-1	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A
Endrin Aldehyde	7421-93-4	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A

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			ER		AR							
			Agonist	Antagonist	Agonist	Antagonist						
<u>NEGATIVE (cont.):</u>												
Epichlorohydrin	106-89-8	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A
Famphur	52-85-7	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A
Heptachlor	76-44-8	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A
Hexachlobenzene	118-74-1	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A
2-MethylNaphthalene	91-57-6	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A
Mirex	2385-85-5	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A
Naphthalene	91-20-3	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A
1,2 Naphthoquinone	524-42-5	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A
Perylene	198-55-0	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A
β-Pinene	127-91-3	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A
Silvex	93-72-1	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A
Taxifolin	480-18-2	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A
p-Xylene	106-42-3	X	X	X	X	X	-	-	ND	Non-Active	N/A	N/A