

Annex K2

Phase 1 Experiments Conducted to Establish Historical Databases

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Table 1 Phase 1 Agonist Plates Tested at XDS

Plate I.D.	Date	DMSO	Induction ^a	EC ₅₀ (µg/mL)	MET ^b	Was Plate Used for Data Analysis?
XICT1BPA	01-Nov-07	2672	3.7	2.8 x 10 ⁻⁶	6796	Yes
XICT2BPA	01-Nov-07	2007	4.9	1.9 x 10 ⁻⁶	5990	Yes
XICT3BPA	02-Nov-07	6619	4.1	2.0 x 10 ⁻⁶	7155	Yes
XICT4BPA	02-Nov-07	5619	5.3	5.8 x 10 ⁻⁶	5065	Yes
XICT5BPA	02-Nov-07	5233	5.3	2.7 x 10 ⁻⁶	4648	Yes
XICT6BPA	02-Nov-07	5841	5.8	2.4 x 10 ⁻⁶	4077	Yes
XICT7BPA	02-Nov-07	7017	3.9	1.7 x 10 ⁻⁶	6480	Yes
XICT8BPA	02-Nov-07	10138	4.1	2.1 x 10 ⁻⁶	5720	Yes
XICT9BPA	08-Aug-07	6758	4.7	2.2 x 10 ⁻⁶	5158	Yes
XICT10BPA	08-Aug-07	2040	4.9	3.1 x 10 ⁻⁶	5999	Yes

Abbreviations: DMSO = dimethyl sulfoxide; EC₅₀ = the half maximal effective concentration; MET = methoxychlor;
XDS = Xenobiotic Detection Systems, Inc.

^a Induction for historical database plates is measured by dividing the averaged highest E2 reference standard RLU value by the averaged DMSO control relative light unit (RLU) value.

^b Methoxychlor measurement is mean adjusted RLU.

Table 2 Phase 1 Agonist Plates Tested at ECVAM

Plate I.D.	Date	DMSO	Induction ^a	EC ₅₀ (µg/mL)	MET ^b	Was Plate Used for Data Analysis?
Ag09112007-001	09-Nov-07	4607	7.7	2.1 x 10 ⁻⁶	5245	Yes
Ag09112007-002	09-Nov-07	4007	9.3	2.7 x 10 ⁻⁶	5072	Yes
Ag13112007-001	13-Nov-07	1379	8.1	2.8 x 10 ⁻⁶	4098	Yes
Ag13112007-002	13-Nov-07	1254	10.1	2.3 x 10 ⁻⁶	4657	Yes
Ag16112007-001	16-Nov-07	3246	8.8	2.1 x 10 ⁻⁶	4482	Yes
Ag16112007-002	16-Nov-07	3552	7.3	1.9 x 10 ⁻⁶	3907	Yes
Ag19112007-001	19-Nov-07	3264	7.3	2.7 x 10 ⁻⁶	4160	Yes
Ag19112007-002	19-Nov-07	3696	6.7	2.5 x 10 ⁻⁶	4799	Yes
Ag22112007-001	22-Nov-07	3150	7.8	3.7 x 10 ⁻⁶	3763	Yes
Ag22112007-002	22-Nov-07	2732	7.4	4.1 x 10 ⁻⁶	3929	Yes
Ag25112007-001	25-Nov-07	1985	8.4	3.5 x 10 ⁻⁶	4725	Yes
Ag25112007-002	25-Nov-07	1999	9.0	3.6 x 10 ⁻⁶	5535	Yes
Ag28112007-001	28-Nov-07	2560	8.1	3.3 x 10 ⁻⁶	3725	Yes
Ag28112007-002	28-Nov-07	2617	6.9	3.2 x 10 ⁻⁶	5052	Yes
Ag01122007-001	01-Dec-07	4829	8.7	3.4 x 10 ⁻⁶	3852	Yes
Ag01122007-002	01-Dec-07	4287	8.8	3.1 x 10 ⁻⁶	5210	Yes
Ag04122007-001	04-Dec-07	6342	7.9	3.5 x 10 ⁻⁶	3882	Yes
Ag04122007-002	04-Dec-07	7236	6.9	3.5 x 10 ⁻⁶	4802	Yes

Abbreviations: DMSO = dimethyl sulfoxide; EC₅₀ = the half maximal effective concentration; ECVAM = European Centre for the Validation of Alternative Methods; MET = methoxychlor.

^a Induction for historical database plates is measured by dividing the averaged highest E2 reference standard RLU value by the averaged DMSO control RLU value.

^b Methoxychlor measurement is mean adjusted RLU.

Table 3 Phase 1 Agonist Plates Tested at Hiyoshi

Plate I.D.	Date	DMSO	Induction ^a	EC ₅₀ (µg/mL)	MET ^b	Was Plate Used for Data Analysis?
HIrefsubAg1	28-May-07	2516	5.2	2.7 x 10 ⁻⁶	7742	Yes
HIrefsubAg2	30-May-07	2323	□4.8	□3.8 x 10 ⁻⁶	8261	Yes
HIrefsubAg3	04-Jun-07	9249	□1.6	4.9 x 10 ⁻⁶	8381	No ^c
HIrefsubAg4	06-Jun-07	5417	□2.7	5.5 x 10 ⁻⁶	7739	No ^c
HIrefsubAg5	11-Jun-07	6087	□3.5	□2.8 x 10 ⁻⁶	8345	Yes
HIrefsubAg6	13-Jun-07	3404	□3.9	2.7 x 10 ⁻⁶	7623	Yes
HIrefsubAg7	18-Jun-07	4873	□3.8	3.6 x 10 ⁻⁶	8497	Yes
HIrefsubAg8	20-Jun-07	3776	□5.0	2.2 x 10 ⁻⁶	7847	Yes
HIrefsubAg9	25-Jun-07	4910	□4.0	4.1 x 10 ⁻⁶	7725	Yes
HIrefsubAg10	27-Jun-07	4686	□3.8	1.8 x 10 ⁻⁶	7654	Yes
HIrefsubAg11	02-Jul-07	5779	□4.5	2.8 x 10 ⁻⁶	7133	Yes
HIrefsubAg12	06-Jul-07	1709	□6.3	□4.2 x 10 ⁻⁶	8342	Yes

Abbreviations: DMSO = dimethyl sulfoxide; EC₅₀ = the half maximal effective concentration; Hiyoshi = Hiyoshi Corporation; MET = methoxychlor.

^a Induction for historical database plates is measured by dividing the averaged highest E2 reference standard RLU value by the averaged DMSO control RLU value.

^b Methoxychlor measurement is mean adjusted RLU.

^c Failed induction.

Table 4 Phase 1 Antagonist Plates Tested at XDS

Plate I.D.	Date	DMSO	Reduction ^a	IC ₅₀ (µg/mL)	E2 Control ^b	Fla\E2 Control ^b	Was Plate Used for Data Analysis?
XICTAnt1.xls	21-Dec-07	242	19.62	4.65 x 10 ⁻⁴	7018	1717	Yes
XICTAnt2.xls	21-Dec-07	190	15.98	5.18 x 10 ⁻⁴	7629	2041	Yes
XICTAnt3.xls	22-Dec-07	na	na	na	na	na	No ^c
XICTAnt4.xls	11-Jan-08	148	14.01	4.07 x 10 ⁻⁴	7795	5730	Yes
XICTAnt5.xls	11-Jan-08	154	15.230	4.43 x 10 ⁻⁴	8206	4332	Yes
XICTAnt6.xls	11-Jan-08	152	13.08	3.89 x 10 ⁻⁴	8799	3258	Yes
XICTAnt7.xls	12-Jan-08	130	14.33	2.87 x 10 ⁻⁴	7493	2673	Yes
XICTAnt8.xls	12-Jan-08	121	14.61	4.07 x 10 ⁻⁴	7423	3096	Yes
XICTAnt9.xls	17-Jan-08	515	15.46	3.67 x 10 ⁻⁴	8827	3986	Yes
XICTAnt10.xls	17-Jan-08	593	12.07	3.33 x 10 ⁻⁴	8642	4312	Yes
XICTAnt11.xls	18-Jan-08	446	15.20	4.29 x 10 ⁻⁴	9763	4372	Yes
XICTAnt12.xls	18-Jan-08	515	12.67	2.91 x 10 ⁻⁴	8790	4722	Yes
XICTAnt13.xls	21-Jan-08	1331	11.85	5.71 x 10 ⁻⁴	8099	2858	Yes
XICTAnt14.xls	21-Jan-08	1193	9.43	4.86 x 10 ⁻⁴	8403	3722	Yes
XICTAnt15.xls	21-Jan-08	1207	15.13	5.65 x 10 ⁻⁴	7908	3338	Yes

Abbreviations: DMSO = dimethyl sulfoxide; E2 = 17β-estradiol; Fla = flavone; IC₅₀ = the half maximal inhibitory concentration; na = not available; XDS = Xenobiotic Detection Systems, Inc.

^a Reduction for historical database plates is measured by dividing the averaged highest Ral/E2 reference standard RLU value by the lowest averaged Ral/E2 reference standard RLU value.

^b Measurement is mean adjusted RLU.

^c Plate contaminated.

Table 5 Phase 1 Antagonist Plates Tested at ECVAM

Plate I.D.	Date	DMSO	Reduction ^a	IC ₅₀ (µg/mL)	E2 Control ^b	Fla\E2 Control ^b	Was Plate Used for Data Analysis?
Ag09112007-001	09-Nov-07	5717	7.5	6.1 x 10 ⁻⁴	7995	-68	Yes
Ag09112007-002	09-Nov-07	3878	8.9	4.4 x 10 ⁻⁴	8484	23	Yes
Ag13112007-001	13-Nov-07	1490	7.9	4.0 x 10 ⁻⁴	8872	1164	Yes
Ag13112007-002	13-Nov-07	1523	8.3	4.4 x 10 ⁻⁴	7239	1181	Yes
Ag16112007-001	16-Nov-07	3625	6.7	5.7 x 10 ⁻⁴	7904	633	Yes
Ag16112007-002	16-Nov-07	3964	8.1	4.2 x 10 ⁻⁴	8354	411	Yes
Ag19112007-001	19-Nov-07	4149	7.4	5.5 x 10 ⁻⁴	8916	1003	Yes
Ag19112007-002	19-Nov-07	4705	6.7	4.8 x 10 ⁻⁴	9214	371	Yes
Ag22112007-001	22-Nov-07	2730	8.1	4.3 x 10 ⁻⁴	9212	1657	Yes
Ag22112007-002	22-Nov-07	2696	7.8	4.2 x 10 ⁻⁴	8958	1024	Yes
Ag25112007-001	25-Nov-07	2393	8.7	4.0 x 10 ⁻⁴	8637	503	Yes
Ag25112007-002	25-Nov-07	2737	9.2	3.8 x 10 ⁻⁴	8605	614	Yes
Ag28112007-001	28-Nov-07	2597	8.3	3.5 x 10 ⁻⁴	9762	670	Yes
Ag28112007-002	28-Nov-07	2801	8.9	3.8 x 10 ⁻⁴	10054	1118	Yes
Ag01122007-001	01-Dec-07	5026	7.7	3.6 x 10 ⁻⁴	8304	135	Yes
Ag01122007-002	01-Dec-07	4726	7.8	3.1 x 10 ⁻⁴	8857	884	Yes
Ag04122007-001	04-Dec-07	6011	7.6	3.9 x 10 ⁻⁴	10203	755	Yes
Ag04122007-002	04-Dec-07	7333	7.5	4.0 x 10 ⁻⁴	9040	659	Yes

Abbreviations: DMSO = dimethyl sulfoxide; E2 = 17β-estradiol; ECVAM = European Centre for the Validation of Alternative Methods; Fla = flavone; IC₅₀ = the half maximal inhibitory concentration.

^a Reduction for historical database plates is measured by dividing the averaged highest Ral/E2 reference standard RLU value by the lowest averaged Ral/E2 reference standard RLU value.

^b Measurement is mean adjusted RLU.

Table 6 Phase 1 Antagonist Plates Tested at Hiyoshi

Plate I.D.	Date	DMSO	Reduction ^a	IC ₅₀ (µg/mL)	E2 Control ^b	Fla\E2 Control ^b	Was Plate Used for Data Analysis?
HlrefsubAnt1	28-May-07	2873	10.7	5.1 x 10 ⁻⁴	5500	425	Yes
HlrefsubAnt2	28-May-07	2155	□6.6	5.9 x 10 ⁻⁴	7608	1772	Yes
HlrefsubAnt3	04-Jun-07	4260	□8.0	7.4 x 10 ⁻⁴	7576	1461	Yes
HlrefsubAnt4	06-Jun-07	4887	□4.3	6.2 x 10 ⁻⁴	5642	2938	Yes
HlrefsubAnt5	11-Jun-07	6541	□6.0	7.9 x 10 ⁻⁴	6150	259	Yes
HlrefsubAnt6	13-Jun-07	3390	□10.0	5.4 x 10 ⁻⁴	5407	760	Yes
HlrefsubAnt7	18-Jun-07	4622	□5.9	5.9 x 10 ⁻⁴	5771	2038	Yes
HlrefsubAnt8	20-Jun-07	4293	□11.4	5.5 x 10 ⁻⁴	4903	705	Yes
HlrefsubAnt9	25-Jun-07	4503	□7.0	7.7 x 10 ⁻⁴	4837	936	Yes
HlrefsubAnt10	27-Jun-07	3875	□6.6	7.7 x 10 ⁻⁴	5338	1660	Yes
HlrefsubAnt11	02-Jul-07	5557	□7.0	7.1 x 10 ⁻⁴	4861	1014	Yes
HlrefsubAnt12	06-Jul-07	1625	□11.0	3.7 x 10 ⁻⁴	9026	1173	Yes

Abbreviations: DMSO = dimethyl sulfoxide; E2 = 17β-estradiol; Hiyoshi = Hiyoshi Corporation; Fla = flavone; IC₅₀ = the half maximal inhibitory concentration.

^a Reduction for historical database plates is measured by dividing the averaged highest Ral/E2 reference standard RLU value by the lowest averaged Ral/E2 reference standard RLU value.

^b Measurement is mean adjusted RLU.