

**Annex K3**  
**Phase 2a Experiments**

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**Table 1 Phase 2a Agonist Range Finder Plates Tested at XDS**

Experiment I.D.	Date	Induction <sup>a</sup>	DMSO	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used
XIIaAgRFX1-X4	04-Apr-08	3.25	1250	Yes	-

<sup>a</sup> Induction for range finder plates is measured by dividing the averaged highest E2 reference standard RLU value by the averaged DMSO control RLU value.

**Table 2 Phase 2a Agonist Range Finder Plates Tested at ECVAM**

Experiment I.D.	Date	Induction <sup>a</sup>	DMSO	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used
ECVAMRf Ag IlaCorrected	16-Apr-08	8.34	2326	Yes	-
Rf Ag Ila 2nd Run	23-Apr-08	1.64	10762	No	Failed Induction

<sup>a</sup> Induction for range finder plates is measured by dividing the averaged highest E2 reference standard RLU value by the averaged DMSO control RLU value.

**Table 3 Phase 2a Agonist Range Finder Plates Tested at Hiyoshi**

Experiment I.D.	Date	Induction <sup>a</sup>	DMSO	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used
AgRF080412 Corrected	12-Apr-08	4.85	2323	Yes	-

<sup>a</sup> Induction for range finder plates is measured by dividing the averaged highest E2 reference standard RLU value by the averaged DMSO control RLU value.

**Table 4 Phase 2a Antagonist Range Finder Plates Tested at XDS**

Experiment I.D.	Date	Reduction <sup>a</sup>	DMSO	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used
XIIaAntRFX5-X8	04-Apr-08	5.33	744	No	Failed Viability

<sup>a</sup> Reduction for range finder plates is measured by dividing the averaged highest Ral/E2 reference standard RLU value by the averaged DMSO control RLU value.

**Table 5 Phase 2a Antagonist Range Finder Plates Tested at ECVAM**

Experiment I.D.	Date	Reduction <sup>a</sup>	DMSO	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used
Rf Anta Ila	16-Apr-08	2.71	9349	No	Failed Reduction <sup>b</sup>
Rf Anta Ila 2 <sup>nd</sup> Run	23-Apr-08	7.51	2759x	Yes	-

<sup>1</sup> Reduction for range finder plates is measured by dividing the averaged highest Ral/E2 reference standard RLU value by the averaged DMSO control RLU value.

<sup>2</sup> Power failure in laboratory

**Table 6 Phase 2a Antagonist Range Finder Plates Tested at Hiyoshi**

Experiment I.D.	Date	Reduction <sup>a</sup>	DMSO	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used
AntRF080412 corrected	12-Apr-08	6.99	2728	Yes	-

<sup>a</sup> Reduction for range finder plates is measured by dividing the averaged highest Ral/E2 reference standard RLU value by the averaged DMSO control RLU value.

**Table 7 Phase 2a Agonist Comprehensive Test Plates Tested at XDS**

Test Substance	Date	DMSO	Induction <sup>a</sup>	E2 Reference Standard EC <sub>50</sub> (µg/mL)	MET	Test Substance Dilution	Test Substance EC <sub>50</sub> (µg/mL)	Positive Using Threshold Method	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
BPA	11-Apr-08	885	5.94	4.02 x 10 <sup>-6</sup>	4389	1:2	1.1 x 10 <sup>-1</sup>	Positive	No	Failed EC <sub>50</sub> Acceptance Criteria	XIIaX0003-X0004CTAg1
BPA	25-Apr-07	1479	6.16	3.96 x 10 <sup>-6</sup>	6285	1:5	9.8 x 10 <sup>-2</sup>	Positive	No	Failed EC <sub>50</sub> Acceptance Criteria	XIIaX0003-X0004CTAg2
BPA	30-Apr-08	503	6.00	3.45 x 10 <sup>-6</sup>	6300	1:5	1.0 x 10 <sup>-1</sup>	Positive	No	Failed EC <sub>50</sub> Acceptance Criteria	XIIaX0003-X0004CTAg3
BPA	02-May-08	637	5.28	3.13 x 10 <sup>-6</sup>	5343	1:5	8.6 x 10 <sup>-2</sup>	Positive	Yes	-	XIIaX0003-X0004CTAg4
BPA	27-Jun-08	1699	5.59	2.21 x 10 <sup>-6</sup>	5773	1:5	8.18 x 10 <sup>-2</sup>	Positive	Yes	-	XIIaX0003-X0004CTAg5v2
BPA	03-Jul-08	3900	3.21	2.76 x 10 <sup>-6</sup>	4137	1:5	9.63 x 10 <sup>-2</sup>	Positive	Yes	-	XIIaX0003-X0004CTAg6v2
BPA	04-Jul-08	1081	10.38	3.41 x 10 <sup>-6</sup>	4388	1:5	7.67 x 10 <sup>-2</sup>	Positive	No	Failed EC <sub>50</sub> Acceptance Criteria	XIIaX0003-X0004CTAg7v2
BPB	11-Apr-08	885	5.94	4.02 x 10 <sup>-6</sup>	4389	1:2	3.3 x 10 <sup>-2</sup>	Positive	No	Failed EC <sub>50</sub> Acceptance Criteria	XIIaX0003-X0004CTAg1
BPB	25-Apr-07	1479	6.16	3.96 x 10 <sup>-6</sup>	6285	1:5	3.5 x 10 <sup>-2</sup>	Positive	No	Failed EC <sub>50</sub> Acceptance Criteria	XIIaX0003-X0004CTAg2
BPB	30-Apr-08	503	6.00	3.45 x 10 <sup>-6</sup>	6300	1:5	4.8 x 10 <sup>-2</sup>	Positive	No	Failed EC <sub>50</sub> Acceptance Criteria	XIIaX0003-X0004CTAg3
BPB	02-May-08	637	5.28	3.13 x 10 <sup>-6</sup>	5343	1:5	3.87 x 10 <sup>-2</sup>	Positive	Yes	-	XIIaX0003-X0004CTAg4
BPB	27-Jun-08	1699	5.59	2.21 x 10 <sup>-6</sup>	5773	1:5	3.27 x 10 <sup>-2</sup>	Positive	Yes	-	XIIaX0003-X0004CTAg5v2

Test Substance	Date	DMSO	Induction <sup>a</sup>	E2 Reference Standard EC <sub>50</sub> (µg/mL)	MET	Test Substance Dilution	Test Substance EC <sub>50</sub> (µg/mL)	Positive Using Threshold Method	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
BPB	03-Jul-08	3900	3.21	2.76 x 10 <sup>-6</sup>	4137	1:5	4.51 x 10 <sup>-2</sup>	Positive	Yes	-	XIIaX0003-X0004CTAg6v2
BPB	04-Jul-08	1081	10.38	3.41 x 10 <sup>-6</sup>	4388	1:5	5.50 x 10 <sup>-2</sup>	Positive	No	Failed EC <sub>50</sub> Acceptance Criteria	XIIaX0003-X0004CTAg7v2
CORT	11-Apr-08	1198	4.05	2.75 x 10 <sup>-6</sup>	4852	1:2	-	Negative	Yes	-	XIIaX0001-X0002CTAg1
CORT	25-Apr-08	1638	4.65	4.26 x 10 <sup>-6</sup>	6190	1:5	-	Negative	No	Failed EC <sub>50</sub> Acceptance Criteria	XIIaX0001-X0002CTAg2
CORT	30-Apr-08	602	5.22	4.47 x 10 <sup>-6</sup>	5330	1:5	-	Negative	No	Failed EC <sub>50</sub> Acceptance Criteria	XIIaX0001-X0002CTAg3
CORT	02-May-08	622	6.22	4.48 x 10 <sup>-6</sup>	3897	1:5	-	Negative	No	Failed EC <sub>50</sub> Acceptance Criteria	XIIaX0001-X0002CTAg4
CORT	26-Jun-08	5114	6.40	2.22 x 10 <sup>-6</sup>	5257	1:5	not calculated	Positive	Yes	-	XIIaX0001-X0002CTAg5v2
CORT	27-Jun-08	2196	4.14	2.59 x 10 <sup>-6</sup>	5797	1:5	-	Negative	Yes	-	XIIaX0001-X0002CTAg6v2
CORT	10-Jul-08	629	14.28	4.17 x 10 <sup>-6</sup>	4987	1:5	not calculated	Positive	No	Failed EC <sub>50</sub> Acceptance Criteria	XIIaX0001-X0002CTAg7
DES	11-Apr-08	1198	4.05	2.75 x 10 <sup>-6</sup>	4852	1:2	1.78 x 10 <sup>-5</sup>	Positive	No	Different DES dilutions.	XIIaX0001-X0002CTAg1
DES	25-Apr-08	1638	4.65	4.26 x 10 <sup>-6</sup>	6190	1:5	3.84 x 10 <sup>-5</sup>	Positive	No	Failed EC <sub>50</sub> Acceptance Criteria	XIIaX0001-X0002CTAg2
DES	30-Apr-08	602	5.22	4.47 x 10 <sup>-6</sup>	5330	1:5	1.51 x 10 <sup>-5</sup>	Positive	No	Failed EC <sub>50</sub> Acceptance Criteria	XIIaX0001-X0002CTAg3
DES	02-May-08	622	6.22	4.48 x 10 <sup>-6</sup>	3897	1:5	3.14 x 10 <sup>-5</sup>	Positive	No	Failed EC <sub>50</sub> Acceptance Criteria	XIIaX0001-X0002CTAg4

Test Substance	Date	DMSO	Induction <sup>a</sup>	E2 Reference Standard EC <sub>50</sub> (µg/mL)	MET	Test Substance Dilution	Test Substance EC <sub>50</sub> (µg/mL)	Positive Using Threshold Method	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
DES	26-Jun-08	5114	6.40	2.22 x 10 <sup>-6</sup>	5257	1:5	1.91 x 10 <sup>-5</sup>	Positive	Yes	-	XIIaX0001-X0002CTAg5v2
DES	27-Jun-08	2196	4.14	2.59 x 10 <sup>-6</sup>	5797	1:5	9.10 x 10 <sup>-6</sup>	Positive	Yes	-	XIIaX0001-X0002CTAg6v2
DES	10-Jul-08	629	14.28	4.17 x 10 <sup>-6</sup>	4987	1:5	1.76 x 10 <sup>-5</sup>	Positive	No	Failed EC <sub>50</sub> Acceptance Criteria	XIIaX0001-X0002CTAg7
DES	19-Jul-08	1080	9.87	3.31 x 10 <sup>-6</sup>	7301	1:5	1.10 x 10 <sup>-5</sup>	Positive	Yes	-	XIIaX0001 Ag8

Abbreviations: BPA = Bisphenol A; BPB = Bisphenol B; CORT = Corticosterone; DES = Diethylstilbestrol; DMSO = Dimethyl sulfoxide; EC<sub>50</sub> = half-maximal effective concentration; E2 = 17β-estradiol; I.D. = Identification; MET = Methoxychlor.

<sup>a</sup> Induction for comprehensive test plates is measured by dividing the averaged highest E2 reference standard RLU value by the averaged DMSO control RLU value.

**Table 8 Phase 2a Agonist Comprehensive Test Plates Tested at ECVAM**

Test Substance	Date	DMSO	Induction <sup>a</sup>	E2 Reference Standard EC <sub>50</sub> (µg/mL)	MET	Test Substance Dilution	Test Substance EC <sub>50</sub> (µg/mL)	Positive Using Threshold Method	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
BPA	30-Apr-08	1189	9.22	6.56 x 10 <sup>-6</sup>	4447	1:2	1.8 x 10 <sup>-1</sup>	Positive	No	Failed EC <sub>50</sub> Acceptance Criterion	Ag CT1 0001 0002
BPA	07-May-08	1451	9.12	5.28 x 10 <sup>-6</sup>	4171	1:2	1.6 x 10 <sup>-1</sup>	Positive	No	Failed EC <sub>50</sub> Acceptance Criterion	Ag CT2 0001 0002
BPA	14-May-08	8313	1.82	6.42 x 10 <sup>-6</sup>	6121	1:2	1.9 x 10 <sup>-1</sup>	Positive	No	Failed DMSO, EC <sub>50</sub> , and Induction Acceptance Criteria	AgCT3 0001 0002
BPA	21-May-08	1640	8.39	7.59 x 10 <sup>-6</sup>	5252	1:2	1.8 x 10 <sup>-1</sup>	Positive	No	Failed EC <sub>50</sub> Acceptance Criterion	AgCT4 0001 0002
BPA	02-Jul-08	7703	1.34	3.83 x 10 <sup>-7</sup>	-1533	1:2	Abnormal conc.-response curve.	-	No	Failed DMSO, EC <sub>50</sub> , Induction, and MET Acceptance Criteria	AgCT5 0001 0002
BPA	09-Jul-08	1522	8.81	3.29 x 10 <sup>-6</sup>	5068	1:2	1.9 x 10 <sup>-1</sup>	Positive	Yes	-	AgCT6 0001 0002
BPA	09-Jul-08	1539	8.43	Positive but could not be calculated	4879	1:2	1.7 x 10 <sup>-1</sup>	Positive	No	Failed EC50 Acceptance Criteria	AgCT7 0001 0002
BPA	06-Aug-08	1415	12.10	2.03 x 10 <sup>-6</sup>	6860	1:2	2.9 x 10 <sup>-1</sup>	Positive	No	Failed MET Acceptance Criteria	AgCT8 0001 0002
BPA	06-Aug-08	1080	11.52	1.23 x 10 <sup>-6</sup>	5434	1:2	1.5 x 10 <sup>-1</sup>	Positive	No	Failed EC50 Acceptance Criteria	AgCT8 B 0001 0002
BPA	13-Aug-08	1502	10.88	2.38 x 10 <sup>-6</sup>	5256	1:2	1.8 x 10 <sup>-1</sup>	Positive	Yes		AgCT9 0001 0002



Test Substance	Date	DMSO	Induction <sup>a</sup>	E2 Reference Standard EC <sub>50</sub> (µg/mL)	MET	Test Substance Dilution	Test Substance EC <sub>50</sub> (µg/mL)	Positive Using Threshold Method	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
BPA	20-Aug-08	5401	5.99	1.70 x 10 <sup>-6</sup>	6073	1:2	7.6 x 10 <sup>-2</sup>	Positive	No	Failed MET Acceptance Criteria	AgCT10 0001 0002
BPA	27-Aug-08	10494	2.44	2.19 x 10 <sup>-6</sup>	1574	1:2	-	Negative	No	Failed DMSO and Induction Acceptance Criteria	AgCT11 0001 0002
BPA	03-Sep-08	6139	3.28	7.00 x 10 <sup>-6</sup>	5697	1:2	1.6 x 10 <sup>-1</sup>	Positive	No	Failed EC50 Acceptance Criteria	AgCT12A 0001 0002
BPA	03-Sep-08	2607	8.08	Positive but could not be calculated	5590	1:2	1.7 x 10 <sup>-1</sup>	Positive	No	Failed EC50 Acceptance Criteria	AgCT12B 0001 0002
BPA	10-Sep-08	4315	7.85	1.67 x 10 <sup>-6</sup>	5841	1:2	1.9 x 10 <sup>-1</sup>	Positive	Yes	-	AgCT13A 0001 0002
BPA	10-Sep-08	5302	6.09	1.67 x 10 <sup>-6</sup>	6346	1:2	1.9 x 10 <sup>-1</sup>	Positive	No	Failed MET Acceptance Criteria	AgCT13B 0001 0002
BPB	30-Apr-08	979	11.77	5.12 x 10 <sup>-6</sup>	4513	1:5	5.85 x 10 <sup>-2</sup>	Positive	No	Failed EC <sub>50</sub> Acceptance Criteria	Ag CT1 0003 0004
BPB	07-May-08	828	8.21	3.94 x 10 <sup>-6</sup>	4303	1:5	5.34 x 10 <sup>-2</sup>	Positive	Yes	-	Ag CT2 0003 0004
BPB	14-May-08	7005	2.82	6.58 x 10 <sup>-6</sup>	1306	1:5	6.80 x 10 <sup>-2</sup>	Positive	No	Failed DMSO, EC <sub>50</sub> , and Induction Acceptance Criteria	AgCT3 0003 0004
BPB	21-May-08	1701	7.42	4.96 x 10 <sup>-6</sup>	5568	1:5	8.77 x 10 <sup>-2</sup>	Positive	No	Failed EC <sub>50</sub>	AgCT4 0003 0004
BPB	02-Jul-08	2304	4.72	3.34 x 10 <sup>-7</sup>	6860	1:5	9.10 x 10 <sup>-2</sup>	Positive	No	Failed EC <sub>50</sub> and MET Acceptance Criteria	AgCT5 0003 0004

Test Substance	Date	DMSO	Induction <sup>a</sup>	E2 Reference Standard EC <sub>50</sub> (µg/mL)	MET	Test Substance Dilution	Test Substance EC <sub>50</sub> (µg/mL)	Positive Using Threshold Method	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
BPB	07-Jul-08	4229	3.40	5.61 x 10 <sup>-6</sup>	6776	1:5	6.03 x 10 <sup>-2</sup>	Positive	No	Failed EC <sub>50</sub> and MET Acceptance Criteria, Abnormal E2 Reference Standard Curve	AgCT6 0003 0004
BPB	09-Jul-08	1577	11.72	1.11 x 10 <sup>-6</sup>	4344	1:5	not calculated	Positive	No	Failed EC50 Acceptance Criteria	AgCT7 0003 0004
BPB	09-Jul-08	1184	9.30	6.61 x 10 <sup>-7</sup>	5426	1:5	not calculated	Positive	No	Failed EC50 Acceptance Criteria	AgCT7 B 0003 0004
BPB	13-Aug-08	7520	2.16	2.80 x 10 <sup>-6</sup>	2908	1:5	1.99 x 10 <sup>-2</sup>	Positive	No	Failed DMSO, Induction	AgCT 8 0003 0004
BPB	20-Aug-08	10768	3.16	1.13 x 10 <sup>-6</sup>	6097	1:5	3.55 x 10 <sup>-2</sup>	Positive	No	Failed DMSO, EC <sub>50</sub> , MET Acceptance Criteria	AgCT9 0003 0004
BPB	27-Aug-08	4389	5.33	2.89 x 10 <sup>-6</sup>	5356	1:5	4.50 x 10 <sup>-2</sup>	Positive	Yes	-	AgCT10 0003 0004
BPB	03-Sep-08	12393	1.58	6.53 x 10 <sup>-6</sup>	4271	1:5	5.15 x 10 <sup>-2</sup>	Positive	No	Failed DMSO, Induction	AgCT11B 0003 0004
BPB	10-Sep-08	9886	3.46	2.21 x 10 <sup>-6</sup>	7101	1:5	1.44 x 10 <sup>-2</sup>	Positive	No	Failed DMSO, MET	AgCT12A 0003 0004
BPB	10-Sep-08	5017	6.96	4.78 x 10 <sup>-6</sup>	5340	1:5	2.84 x 10 <sup>-2</sup>	Positive	Yes	-	AgCT12B 0003 0004
CORT	30-Apr-08	1189	9.22	6.56 x 10 <sup>-6</sup>	4447	1:2	9.05	Positive	No	Failed EC <sub>50</sub> Acceptance Criteria	Ag CT1 0001 0002

Test Substance	Date	DMSO	Induction <sup>a</sup>	E2 Reference Standard EC <sub>50</sub> (µg/mL)	MET	Test Substance Dilution	Test Substance EC <sub>50</sub> (µg/mL)	Positive Using Threshold Method	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
CORT	07-May-08	1451	9.12	5.28 x 10 <sup>-6</sup>	4171	1:2	-	Negative	No	Failed EC <sub>50</sub> Acceptance Criteria	Ag CT2 0001 0002
CORT	14-May-08	8313	1.82	6.42 x 10 <sup>-6</sup>	6121	1:2	-	Negative	No	Failed DMSO, EC <sub>50</sub> , and Induction Acceptance Criteria	AgCT3 0001 0002
CORT	21-May-08	1640	8.39	7.59 x 10 <sup>-6</sup>	5252	1:2	-	Negative	No	Failed EC <sub>50</sub> Acceptance Criterion	AgCT4 0001 0002
CORT	02-July-08	7703	1.34	3.83 x 10 <sup>-7</sup>	-1533	1:2	not calculated	Positive	No	Failed DMSO, EC <sub>50</sub> , Induction, and MET Acceptance Criteria	AgCT5 0001 0002
CORT	09-Jul-08	1522	8.81	3.29 x 10 <sup>-6</sup>	5068	1:2	not calculated	Positive	Yes	-	AgCT6 0001 0002
CORT	09-Jul-08	1539	8.43	Positive but could not be calculated	4879	1:2	Abnormal conc.-response curve	-	No	Failed EC50 Acceptance Criteria	AgCT7 0001 0002
CORT	06-Aug-08	1415	12.10	2.03 x 10 <sup>-6</sup>	6860	1:2	not calculated	Positive	No	Failed MET Acceptance Criteria	AgCT8 0001 0002
CORT	06-Aug-08	1080	11.52	1.23 x 10 <sup>-6</sup>	5434	1:2	not calculated	Positive	No	Failed EC50 Acceptance Criteria	AgCT8 B 0001 0002
CORT	13-Aug-08	1502	10.88	2.38 x 10 <sup>-6</sup>	5256	1:2	10.25	Positive	Yes	-	AgCT9 0001 0002
CORT	20-Aug-08	5401	5.99	1.70 x 10 <sup>-6</sup>	6073	1:2	not calculated	Positive	No	Failed MET Acceptance Criteria	AgCT10 0001 0002

Test Substance	Date	DMSO	Induction <sup>a</sup>	E2 Reference Standard EC <sub>50</sub> (µg/mL)	MET	Test Substance Dilution	Test Substance EC <sub>50</sub> (µg/mL)	Positive Using Threshold Method	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
CORT	27-Aug-08	10494	2.44	2.19 x 10 <sup>-6</sup>	1574	1:2	-	Negative	No	Failed DMSO and Induction Acceptance Criteria	AgCT11 0001 0002
CORT	03-Sep-08	6139	3.28	7.00 x 10 <sup>-6</sup>	5697	1:2	not calculated	Positive	No	Failed EC50 Acceptance Criteria	Ag12CTA 0001 0002
CORT	03-Sep-08	2607	8.08	Positive but could not be calculated	5590	1:2	4.00	Positive	No	Failed EC50 Acceptance Criteria	AgCT12B 0001 0002
CORT	10-Sep-08	4315	7.85	1.67 x 10 <sup>-6</sup>	5841	1:2	not calculated	Positive	Yes	-	AgCT13A 0001 0002
CORT	10-Sep-08	5302	6.09	1.67 x 10 <sup>-6</sup>	6346	1:2	not calculated	Positive	No	Failed MET Acceptance Criteria	AgCT13B 0001 0002
DES	30-Apr-08	979	11.77	5.12 x 10 <sup>-6</sup>	4513	1:5	3.05 x 10 <sup>-5</sup>	Positive	No	Failed EC <sub>50</sub> Acceptance Criteria	Ag CT1 0003 0004
DES	07-May-08	828	8.21	3.94 x 10 <sup>-6</sup>	4303	1:5	4.84 x 10 <sup>-6</sup>	Positive	Yes	-	Ag CT2 0003 0004
DES	14-May-08	7005	2.82	6.58 x 10 <sup>-6</sup>	1306	1:5	1.01 x 10 <sup>-5</sup>	Positive	No	Failed DMSO, EC <sub>50</sub> , and Induction Acceptance Criteria	AgCT3 0003 0004
DES	21-May-08	1701	7.42	4.96 x 10 <sup>-6</sup>	5568	1:5	6.02 x 10 <sup>-6</sup>	Positive	No	Failed EC <sub>50</sub> Acceptance	AgCT4 0003 0004
DES	02-Jul-08	2304	4.72	3.34 x 10 <sup>-7</sup>	6860	1:5	4.68 x 10 <sup>-5</sup>	Positive	No	Failed EC <sub>50</sub> and MET Acceptance Criteria	AgCT5 0003 0004

Test Substance	Date	DMSO	Induction <sup>a</sup>	E2 Reference Standard EC <sub>50</sub> (µg/mL)	MET	Test Substance Dilution	Test Substance EC <sub>50</sub> (µg/mL)	Positive Using Threshold Method	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
DES	7-Jul-08	4229	3.40	5.61 x 10 <sup>-6</sup>	6776	1:5	2.73 x 10 <sup>-5</sup>	Positive	No	Failed EC <sub>50</sub> and MET Acceptance Criteria, Abnormal E2 Reference Standard Curve	AgCT6 0003 0004
DES	09-Jul-08	1577	11.72	1.11 x 10 <sup>-6</sup>	4344	1:5	not calculated	Positive	No	Failed EC50 Acceptance Criteria	AgCT7 0003 0004
DES	09-Jul-08	1184	9.30	6.61 x 10 <sup>-7</sup>	5426	1:5	not calculated	Positive	No	Failed EC50 Acceptance Criteria	AgCT7 B 0003 0004
DES	13-Aug-08	7520	2.16	2.80 x 10 <sup>-6</sup>	2908	1:5	1.32 x 10 <sup>-5</sup>	Positive	No	Failed DMSO, Induction	AgCT 8 0003 0004
DES	20-Aug-08	10768	3.16	1.13 x 10 <sup>-6</sup>	6097	1:5	1.39 x 10 <sup>-5</sup>	Positive	No	Failed DMSO, EC <sub>50</sub> , MET Acceptance Criteria	AgCT9 0003 0004
DES	27-Aug-08	4389	5.33	2.89 x 10 <sup>-6</sup>	5356	1:5	not calculated	Positive	Yes	Used wrong DES concentrations	AgCT10 0003 0004
DES	03-Sep-08	12393	1.58	6.53 x 10 <sup>-6</sup>	4271	1:5	5.8 x 10 <sup>-7</sup>	Positive	No	Failed DMSO, Induction	AgCT11B 0003 0004
DES	10-Sep-08	9886	3.46	2.21 x 10 <sup>-6</sup>	7101	1:5	9.97 x 10 <sup>-6</sup>	Positive	No	Failed DMSO, MET	AgCT12A 0003 0004
DES	10-Sep-08	5017	6.96	4.78 x 10 <sup>-6</sup>	5340	1:5	1.45 x 10 <sup>-5</sup>	Positive	Yes	-	AgCT12B 0003 0004

Abbreviations: BPA = Bisphenol A; BPB = Bisphenol B; CORT = Corticosterone; DES = Diethylstilbestrol; DMSO = Dimethyl sulfoxide; EC<sub>50</sub> = half-maximal effective concentration; E2 = 17β-estradiol; I.D. = Identification; MET = Methoxychlor.

<sup>a</sup> Induction for comprehensive test plates is measured by dividing the averaged highest E2 reference standard RLU value by the averaged DMSO control RLU value.

**Table 9 Phase 2a Agonist Comprehensive Test Plates Tested at Hiyoshi**

Test Substance	Date	DMSO	Induction <sup>a</sup>	E2 Reference Standard EC <sub>50</sub> (µg/mL)	MET	Test Substance Dilution	Test Substance EC <sub>50</sub> (µg/mL)	Positive Using Threshold Method	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
BPA	22-Apr-08	2174	4.00	2.63 x 10 <sup>-6</sup>	8492	1:6	9.00 x 10 <sup>-2</sup>	Positive	Yes	-	AgCT080422 – Ag1
BPA	01-May-08	6272	4.60	3.05 x 10 <sup>-6</sup>	8634	1:6	9.46 x 10 <sup>-2</sup>	Positive	Yes	-	AgCT080501 – Ag 3
BPA	16-May-08	5581	4.51	2.03 x 10 <sup>-6</sup>	10030	1:6	9.28 x 10 <sup>-2</sup>	Positive	No	Failed MET Acceptance Criterion	AgCT080516 – Ag 5
BPA	20-Jun-08	3621	5.85	1.81 x 10 <sup>-6</sup>	8448	1:6	8.61 x 10 <sup>-2</sup>	Positive	Yes	-	AgCT080422 – Ag8
BPB	22-Apr-08	2174	4.00	2.63 x 10 <sup>-6</sup>	8492	1:6	6.11 x 10 <sup>-2</sup>	Positive	Yes	-	AgCT080422 – Ag1
BPB	01-May-08	6272	4.60	3.05 x 10 <sup>-6</sup>	8634	1:6	6.27 x 10 <sup>-2</sup>	Positive	Yes	-	AgCT080501 – Ag 3
BPB	16-May-08	5581	4.51	2.03 x 10 <sup>-6</sup>	10030	1:6	7.30 x 10 <sup>-2</sup>	Positive	No	Failed MET Acceptance Criterion	AgCT080516 – Ag 5
BPB	20-Jun-08	3621	5.85	1.81 x 10 <sup>-6</sup>	8448	1:6	5.91 x 10 <sup>-2</sup>	Positive	Yes	-	AgCT080422 – Ag8
CORT	22-Apr-08	2023	3.82	1.80 x 10 <sup>-6</sup>	8837	1:6	-	Negative	Yes	-	AgCT080422 – Ag2.1
CORT	01-May-08	6314	4.52	2.40 x 10 <sup>-6</sup>	8553	1:6	-	Negative	Yes	-	AgCT080501 – Ag4
CORT	16-May-08	4925	4.31	2.01 x 10 <sup>-6</sup>	8914	1:6	-	Negative	Yes	-	AgCT080516 – Ag6
CORT	31-May-08	4066	3.74	2.58 x 10 <sup>-6</sup>	7622	1:6	-	Negative	Yes	-	AgCT080531 – Ag7
DES	01-May-08	6175	4.42	2.16 x 10 <sup>-6</sup>	8995	1:6	6.78 x 10 <sup>-6</sup>	Positive	Yes	-	AgCT080501 – Ag2.2
DES	01-May-08	6314	4.52	2.40 x 10 <sup>-6</sup>	8553	1:6	7.93 x 10 <sup>-6</sup>	Positive	Yes	-	AgCT080501 – Ag4
DES	16-May-08	4925	4.31	2.01 x 10 <sup>-6</sup>	8914	1:6	3.55 x 10 <sup>-6</sup>	Positive	Yes	-	AgCT080516 – Ag6

Test Substance	Date	DMSO	Induction <sup>a</sup>	E2 Reference Standard EC <sub>50</sub> (µg/mL)	MET	Test Substance Dilution	Test Substance EC <sub>50</sub> (µg/mL)	Positive Using Threshold Method	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
DES	31-May-08	4066	3.74	2.58 x 10 <sup>-6</sup>	7622	1:6	3.95 x 10 <sup>-6</sup>	Positive	Yes	-	AgCT080531 – Ag7

Abbreviations: BPA = Bisphenol A; BPB = Bisphenol B; CORT = Corticosterone; DES = Diethylstilbestrol; DMSO = Dimethyl sulfoxide; EC<sub>50</sub> = half-maximal effective concentration; E2 = 17β-estradiol; I.D. = Identification; MET = Methoxychlor

<sup>a</sup> Induction for comprehensive test plates is measured by dividing the averaged highest E2 reference standard RLU value by the averaged DMSO control RLU value.

**Table 10 Phase 2a Antagonist Plates Tested at XDS**

Test Substance	Date	DMSO	Reduction <sup>1</sup>	Ral/E2 Reference Standard IC <sub>50</sub> (µg/mL)	E2 Cont	Fla/E2 Cont	Test Substance Dilution	Test Substance IC <sub>50</sub> (µg/mL)	Positive Using Threshold Method	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
DBA	11-Apr-08	1255	5.63	7.77 x 10 <sup>-4</sup>	9274	4312	1:2	not calculated	Positive	No	Failed IC <sub>50</sub> Acceptance Criterion	XIIaX0007-X0008CTAnt1
DBA	17-Apr-08	903	6.18	8.08 x 10 <sup>-4</sup>	7205	3260	1:2	-	Negative	No	Failed IC <sub>50</sub> Acceptance Criterion	XIIaX0007-X0008CTAnt2
DBA	18-Apr-08	931	5.16	8.16 x 10 <sup>-4</sup>	8638	5043	1:2	-	Negative	No	Failed IC <sub>50</sub> Acceptance Criterion	XIIaX0007-X0008CTAnt3
DBA	27-Jun-08	1979	11.88	2.85 x 10 <sup>-4</sup>	7846	3022	1:2	not calculated	Positive	Yes	-	XIIaX0007-X0008CTAnt4 v2
DBA	03-Jul-08	1633	11.06	2.83 x 10 <sup>-4</sup>	8824	4245	1:2	not calculated	Positive	Yes	-	XIIaX0007-X0008CTAnt5
DBA	04-Jul-08	1987	11.32	3.62 x 10 <sup>-4</sup>	8053	2661	1:2	not calculated	Positive	Yes	-	XIIaX0007-X0008CTAnt6
NON	11-Apr-08	1255	5.63	7.77 x 10 <sup>-4</sup>	9274	4312	1:2	-	Negative	No	Failed IC <sub>50</sub> Acceptance Criterion	XIIaX0007-X0008CTAnt1
NON	17-Apr-08	903	6.18	8.08 x 10 <sup>-4</sup>	7205	3260	1:2	-	Negative	No	Failed IC <sub>50</sub> Acceptance Criterion	XIIaX0007-X0008CTAnt2
NON	18-Apr-08	931	5.16	8.16 x 10 <sup>-4</sup>	8638	5043	1:2	-	Negative	No	Failed IC <sub>50</sub> Acceptance Criterion	XIIaX0007-X0008CTAnt3
NON	27-Jun-08	1979	11.88	2.85 x 10 <sup>-4</sup>	7846	3022	1:2	-	Negative	Yes	-	XIIaX0007-X0008CTAnt4 v2
NON	03-Jul-08	1633	11.06	2.83 x 10 <sup>-4</sup>	8824	4245	1:2	-	Negative	Yes	-	XIIaX0007-X0008CTAnt5
NON	04-Jul-08	1987	11.32	3.62 x 10 <sup>-4</sup>	8053	2661	1:2	-	Negative	Yes	-	XIIaX0007-X0008CTAnt6



Test Substance	Date	DMSO	Reduction <sup>1</sup>	Ra/E2 Reference Standard IC <sub>50</sub> (µg/mL)	E2 Cont	Fla/E2 Cont	Test Substance Dilution	Test Substance IC <sub>50</sub> (µg/mL)	Positive Using Threshold Method	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
PRO	11-Apr-08	1310	6.99	7.34 x 10 <sup>-4</sup>	9253	3909	1:2	-	Negative	No	Failed IC <sub>50</sub> Acceptance Criterion	XIIaX0005-X0006CTAnt1
PRO	17-Apr-08	724	7.43	7.20 x 10 <sup>-4</sup>	8059	3813	1:2	-	Negative	No	Failed IC <sub>50</sub> Acceptance Criterion	XIIaX0005-X0006CTAnt2
PRO	18-Apr-08	818	7.09	7.72 x 10 <sup>-4</sup>	8393	4057	1:2	-	Negative	No	Failed IC <sub>50</sub> Acceptance Criterion	XIIaX0005-X0006CTAnt3
PRO	27-Jun-08	1671	13.55	3.19 x 10 <sup>-4</sup>	7582	2908	1:2	not calculated	Positive	Yes	-	XIIaX0005-X0006CTAnt4 v2
PRO	03-Jul-08	1980	15.76	3.22 x 10 <sup>-4</sup>	8270	3934	1:2	not calculated	Positive	Yes	-	XIIaX0005-X0006CTAnt5
PRO	04-Jul-08	1550	8.66	3.22 x 10 <sup>-4</sup>	8238	3211	1:2	not calculated	Positive	Yes	-	XIIaX0005-X0006CTAnt6
TAM	11-Apr-08	1310	6.99	7.34 x 10 <sup>-4</sup>	9253	3909	1:5	0.21	Positive	No	Failed IC <sub>50</sub> Acceptance Criterion	XIIaX0005-X0006CTAnt1
TAM	17-Apr-08	724	7.43	7.20 x 10 <sup>-4</sup>	8059	3813	1:5	0.10	Positive	No	Failed IC <sub>50</sub> Acceptance Criterion	XIIaX0005-X0006CTAnt2
TAM	18-Apr-08	818	7.09	7.72 x 10 <sup>-4</sup>	8393	4057	1:5	0.13	Positive	No	Failed IC <sub>50</sub> Acceptance Criterion	XIIaX0005-X0006CTAnt3
TAM	27-Jun-08	1671	13.55	3.19 x 10 <sup>-4</sup>	7582	2908	1:5	not calculated	Positive	Yes	Pipetting Error	XIIaX0005-X0006CTAnt4 v2
TAM	03-Jul-08	1980	15.76	3.22 x 10 <sup>-4</sup>	8270	3934	1:5	0.18	Positive	Yes	-	XIIaX0005-X0006CTAnt5
TAM	04-Jul-08	1550	8.66	3.22 x 10 <sup>-4</sup>	8238	3211	1:5	0.34	Positive	Yes	-	XIIaX0005-X0006CTAnt6
TAM	29-Aug-08	271	8.70	6.30 x 10 <sup>-4</sup>	9556	4385	1:5	0.33	Positive	Yes	-	XIIaX0005CT Ant7

Test Substance	Date	DMSO	Reduction <sup>1</sup>	Ral/E2 Reference Standard IC <sub>50</sub> (µg/mL)	E2 Cont	Fla/E2 Cont	Test Substance Dilution	Test Substance IC <sub>50</sub> (µg/mL)	Positive Using Threshold Method	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
TAM	29-Aug-08	288	7.90	5.10 x 10 <sup>-4</sup>	10061	4590	1:5	0.38	Positive	Yes		XIIaX0005CT Ant8

Abbreviations: Cont = Control; DBA = Dibenzo [*a,h*] anthracene; DMSO = Dimethyl sulfoxide; E2 = 17β-estradiol; Fla = Flavone; IC<sub>50</sub> = half-maximal inhibitory concentration; I.D. = Identification; NON = *p*-n-nonylphenol; PRO = Progesterone; TAM = Tamoxifen

<sup>1</sup> Reduction for comprehensive test plates is measured by dividing the averaged highest Ral/E2 reference standard RLU value by the lowest averaged Ral/E2 reference standard RLU value.

**Table 11 Phase 2a Antagonist Plates Tested at ECVAM**

Test Substance	Date	DMSO	Reduction <sup>1</sup>	Ral/E2 Reference Standard IC <sub>50</sub> (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC <sub>50</sub> (µg/mL)	Positive Using Threshold Method	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
DBA	30-Apr-08	1352	14.74	3.79 x 10 <sup>-4</sup>	9701	1113	1:2	not calculated	Positive	Yes	-	Anta CT1 0081 0082
DBA	07-May-08	1703	9.44	4.51 x 10 <sup>-4</sup>	9300	1311	1:2	not calculated	Positive	Yes	-	Anta CT2 0081 0082
DBA	14-May-08	1575	11.73	5.08 x 10 <sup>-4</sup>	10767	935	1:2	not calculated	Positive	No	Failed E2 Cont Acceptance Criterion	Anta CT3 0081 0082
DBA	25-Jun-08	2953	11.61	4.3 x 10 <sup>-4</sup>	8559	-127	1:2	not calculated	Positive	Yes	-	Anta CT4 0081 0082
NON	30-Apr-08	1378	11.55	3.42 x 10 <sup>-4</sup>	9675	840	1:2	not calculated	Positive	Yes	-	Anta CT1 0079 0080
NON	07-May-08	1509	11.27	3.58 x 10 <sup>-4</sup>	8961	1171	1:2	not calculated	Positive	Yes	-	Anta CT2 0079 0080
NON	14-May-08	1822	11.23	3.89 x 10 <sup>-4</sup>	11302	930	1:2	not calculated	Positive	No	Failed E2 Cont Acceptance Criterion	Anta CT3 0079 0080
NON	25-Jun-08	1862	13.22	5.75 x 10 <sup>-4</sup>	8295	403	1:2	not calculated	Positive	Yes	-	Anta CT4 0079 0080
PRO	30-Apr-08	1378	11.55	3.42 x 10 <sup>-4</sup>	9675	840	1:2	not calculated	Positive	Yes	-	Anta CT1 0079 0080
PRO	07-May-08	1509	11.27	3.58 x 10 <sup>-4</sup>	8961	1171	1:2	not calculated	Positive	Yes	-	Anta CT2 0079 0080
PRO	14-May-08	1822	11.23	3.89 x 10 <sup>-4</sup>	11302	930	1:2	not calculated	Positive	No	Failed E2 Cont Acceptance Criterion	Anta CT3 0079 0080
PRO	25-Jun-08	1862	13.22	5.75 x 10 <sup>-4</sup>	8295	403	1:2	not calculated	Positive	Yes	-	Anta CT4 0079 0080

Test Substance	Date	DMSO	Reduction <sup>1</sup>	Ral/E2 Reference Standard IC <sub>50</sub> (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC <sub>50</sub> (µg/mL)	Positive Using Threshold Method	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
TAM	30-Apr-08	1352	14.74	3.79 x 10 <sup>-4</sup>	9701	1113	1:2	0.17	Positive	Yes	-	Anta CT1 0081 0082
TAM	07-May-08	1703	9.44	4.51 x 10 <sup>-4</sup>	9300	1311	1:2	0.15	Positive	Yes	-	Anta CT2 0081 0082
TAM	14-May-08	1575	11.73	5.08 x 10 <sup>-4</sup>	10767	935	1:2	0.13	Positive	No	Failed E2 Cont Acceptance Criterion	Anta CT3 0081 0082
TAM	25-Jun-08	2953	11.61	4.30 x 10 <sup>-4</sup>	8559	-127	1:2	not calculated	Positive	Yes	Plate used-mistake made during prep. of TAM serial dilutions.	Anta CT4 0081 0082
TAM	09-Jul-08	3031	11.00	4.90 x 10 <sup>-4</sup>	7235	-579	1:2	0.18	Positive	No	Failed Fla\E2 Acceptance Criterion	Anta CT5 0082
TAM	06-Aug-08	16000	11.11	3.53 x 10 <sup>-4</sup>	20345	-227676	1:2	-	Negative	No	Failed DMSO, E2, and Fla\E2 Acceptance Criteria	Anta CT6 0082
TAM	06-Aug-08	4553	11.12	4.07 x 10 <sup>-4</sup>	7247	-5327	1:2	-	Negative	No	Failed Fla\E2 Acceptance Criterion	Anta CT6 B 0082
TAM	13-Aug-08	1714	10.07	6.13 x 10 <sup>-4</sup>	11691	1402	1:2	not calculated	Positive	No	Failed E2 Acceptance Criterion	Anta CT7 0082
TAM	20-Aug-08	5102	12.57	4.60 x 10 <sup>-4</sup>	9747	418	1:2	0.16	Positive	Yes	-	Anta CT8 0082
TAM	27-Aug-08	4831	10.7	5.92 x 10 <sup>-4</sup>	12503	-41	1:2	0.20	Positive	No	Failed E2 Acceptance Criteria	Anta CT9 0082

Abbreviations: Cont = Control; DBA = Dibenzo [*a,h*] anthracene; DMSO = Dimethyl sulfoxide; E2 = 17 $\beta$ -estradiol; Fla = Flavone; IC<sub>50</sub> = half-maximal inhibitory concentration; I.D. = Identification; NON = *p*-n-nonylphenol; PRO = Progesterone; TAM = Tamoxifen

<sup>1</sup> Reduction for comprehensive test plates is measured by dividing the averaged highest Ral/E2 reference standard RLU value by the lowest averaged Ral/E2 reference standard RLU value.

**Table 12 Phase 2a Antagonist Plates Tested at Hiyoshi**

Test Substance	Date	DMSO	Reduction <sup>1</sup>	Ral/E2 Reference Standard IC <sub>50</sub> (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC <sub>50</sub> (µg/mL)	Positive Using Threshold Method	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
DBA	25-Apr-08	4687	7.54	5.51 x 10 <sup>-4</sup>	5636	770	1:2	not calculated	Positive	Yes	-	AntCT080425-Ant1
DBA	09-May-08	3268	15.29	4.96 x 10 <sup>-4</sup>	6143	347	1:2	not calculated	Positive	Yes	-	AntCT080509-Ant3
DBA	21-May-08	6635	10.17	7.83 x 10 <sup>-4</sup>	5368	283	1:2	not calculated	Positive	Yes	-	AntCT080521-Ant5
NON	25-Apr-08	4972	10.65	5.29 x 10 <sup>-4</sup>	5311	713	1:2	not calculated	Positive	Yes	-	AntCT080425-Ant2
NON	09-May-08	2846	15.38	4.99 x 10 <sup>-4</sup>	5824	500	1:2	not calculated	Positive	Yes	-	AntCT080509-Ant4
NON	21-May-08	7221	9.45	8.90 x 10 <sup>-4</sup>	6043	371	1:2	not calculated	Positive	Yes	-	AntCT080521-Ant6
PRO	25-Apr-08	4687	7.54	5.51 x 10 <sup>-4</sup>	5636	770	1:2	-	Negative	Yes	-	AntCT080425-Ant1
PRO	09-May-08	3268	15.29	4.96 x 10 <sup>-4</sup>	6143	347	1:2	-	Negative	Yes	-	AntCT080509-Ant3
PRO	21-May-08	6635	10.17	7.83 x 10 <sup>-4</sup>	5368	283	1:2	not calculated	Positive	Yes	-	AntCT080521-Ant5
TAM	25-Apr-08	4972	10.65	5.29 x 10 <sup>-4</sup>	5495	713	1:2	0.42	Positive	Yes	-	AntCT080425-Ant2
TAM	09-May-08	2846	15.38	4.99 x 10 <sup>-4</sup>	5824	500	1:2	0.32	Positive	Yes	-	AntCT080509-Ant4
TAM	21-May-08	7221	9.45	8.90 x 10 <sup>-4</sup>	6043	371	1:2	0.59	Positive	Yes	-	AntCT080521-Ant6

Abbreviations: Cont = Control; DBA = Dibenzo [*a,h*] anthracene; DMSO = Dimethyl sulfoxide; E2 = 17β-estradiol; Fla = Flavone;

IC<sub>50</sub> = half-maximal inhibitory concentration; I.D. = Identification; NON = *p*-n-nonylphenol; PRO = Progesterone; TAM = Tamoxifen

<sup>1</sup> Reduction for comprehensive test plates is measured by dividing the averaged highest Ral/E2 reference standard RLU value by the lowest averaged Ral/E2 reference standard RLU value.