

Annex K4
Phase IIb Experiments

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Table 1 Phase IIb Agonist Range Finder Plates Tested at XDS

Experiment I.D.	Date	Induction ¹	DMSO	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used
XIIbAgRFX9-X12v3	17-Sep-08	8.12	845	Yes	
XIIbAgRFX13-X16v3	17-Sep-08	11.02	559	Yes	

¹ 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 IIb Agonist Range Finder Plates Tested at ECVAM

Experiment I.D.	Date	Induction ¹	DMSO	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used
AgRf1 V0005 V0008	02-Oct-08	17.61	2326	Yes	
AgRf1 V0009 V0012	02-Oct-08	7.86	5864	Yes	
AgRf2 V0009 V0012	15-Oct-08	6.17	18357	No	Failed DMSO Control
AgRf3 V0009 V0012	22-Oct-08	5.20	15037	No	Failed DMSO Control

¹ 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 IIb Agonist Range Finder Plates Tested at Hiyoshi

Experiment I.D.	Date	Induction ¹	DMSO	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used
AgRF080923-01	23-Sep-08	8.43	2824	Yes	
AgRF080923-02	23-Sep-08	5.92	3343	Yes	

¹ 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 IIb Antagonist Range Finder Plates Tested at XDS

Experiment I.D.	Date	Reduction ¹	DMSO	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used
XIIbAntRFX17-X20v3	17-Sep-08	7.96	852	Yes	
XIIbAntRFX21-X24	18-Sep-08	17.08	676	Yes	

¹ 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 IIb Antagonist Range Finder Plates Tested at ECVAM

Experiment I.D.	Date	Reduction ¹	DMSO	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used
AntaRf4 V0083 V0086	12-Nov-08	6.30	3280	Yes	
AntaRf3 V0087 V0090	12-NOv-08	6.26	2155	Yes	

¹ 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 6 Phase IIb Antagonist Range Finder Plates Tested at Hiyoshi

Experiment I.D.	Date	Reduction ¹	DMSO	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used
AntRF080923-01	23-Sep-08	8.72	4662	Yes	
AntRF080923-02	23-Sep-08	8.40	4577	Yes	

¹ 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 IIb Agonist Comprehensive Test Plates Tested at XDS

Test Substance	Date	DMSO	Induction ¹	E2 Reference Standard EC ₅₀ (µg/mL)	MET	Test Substance Dilution	Test Substance EC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
ATZ	29-Oct-08	1117	8.83	2.89 x 10 ⁻⁶	5751	1:2	Positive but EC ₅₀ value could not be calculated	Yes		XIIb0015-X0016Ag1
ATZ	04-Nov-08	1307	9.85	4.03 x 10 ⁻⁶	6074	1:2	Negative	Yes		XIIb0015-X0016Ag2
ATZ	06-Nov-08	4633	5.71	2.07 x 10 ⁻⁶	7743	1:2	Negative	Yes		XIIb0015-X0016Ag3
BBP	29-Oct-08	1125	6.50	1.81 x 10 ⁻⁶	6131	1:5	2.43 x 10 ⁻¹	Yes		XIIb0013-X0014Ag1
BBP	04-Nov-08	1072	9.65	2.52 x 10 ⁻⁶	5703	1:5	4.58 x 10 ⁻¹	Yes		XIIb0013-X0014Ag2
BBP	06-Nov-08	3305	6.63	3.05 x 10 ⁻⁶	6320	1:5	4.01 x 10 ⁻¹	Yes		XIIb0013-X0014Ag3
DDT	29-Oct-08	628	10.32	3.11 x 10 ⁻⁶	4705	1:2	2.89 x 10 ⁻²	Yes		XIIb0011-X0012CTAg1
DDT	04-Nov-08	1179	9.13	2.64 x 10 ⁻⁶	7595	1:2	2.03 x 10 ⁻²	Yes		XIIb0011-X0012CTAg2
DDT	06-Nov-08	4059	5.56	3.32 x 10 ⁻⁶	6573	1:2	1.59 x 10 ⁻²	Yes		XIIb0011-X0012CTAg3
EE	29-Oct-08	1117	8.83	2.89 x 10 ⁻⁶	5751	1:5	Positive but EC ₅₀ value could not be calculated	Yes	Wrong Test Concentrations	XIIb0015-X0016Ag1
EE	04-Nov-08	1307	9.85	4.03 x 10 ⁻⁶	6074	1:5	2.83 x 10 ⁻⁶	Yes		XIIb0015-X0016Ag2
EE	06-Nov-08	4633	5.71	2.07 x 10 ⁻⁶	7743	1:5	1.32 x 10 ⁻⁶	Yes		XIIb0015-X0016Ag3
EE	13-Nov-08	3093	5.02	1.72 x 10 ⁻⁶	6507	1:5	2.15 x 10 ⁻⁶	Yes		XIIb0016Ag4
FLA	29-Oct-08	1125	6.50	1.81 x 10 ⁻⁶	6131	1:2	Positive but EC ₅₀ value could not be calculated	Yes		XIIb0013-X0014Ag1
FLA	04-Nov-08	1072	9.65	2.52 x 10 ⁻⁶	5703	1:2	Positive but EC ₅₀ value could not be calculated	Yes		XIIb0013-X0014Ag2
FLA	06-Nov-08	3305	6.63	3.05 x 10 ⁻⁶	6320	1:2	Positive but EC ₅₀ value could not be calculated	Yes		XIIb0013-X0014Ag3

Test Substance	Date	DMSO	Induction ¹	E2 Reference Standard EC ₅₀ (µg/mL)	MET	Test Substance Dilution	Test Substance EC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
GEN	29-Oct-08	628	10.32	3.11 x 10 ⁻⁶	4705	1:5	4.54 x 10 ⁻³	Yes		XIIb0011-X0012CTAg1
GEN	04-Nov-08	1179	9.13	2.64 x 10 ⁻⁶	7595	1:5	4.91 x 10 ⁻³	Yes		XIIb0011-X0012CTAg2
GEN	06-Nov-08	4059	5.56	3.32 x 10 ⁻⁶	6573	1:5	7.52 x 10 ⁻³	Yes		XIIb0011-X0012CTAg3
NON	29-Oct-08	816	7.31	2.74 x 10 ⁻⁶	6658	1:5	3.81 x 10 ⁻¹	Yes		XIIb0009-X0010CTAg1
NON	04-Nov-08	1744	4.15	3.68 x 10 ⁻⁶	4818	1:5	4.10 x 10 ⁻¹	Yes		XIIb0009-X0010CTAg2
NON	06-Nov-08	3641	6.20	2.50 x 10 ⁻⁶	5057	1:5	3.87 x 10 ⁻¹	Yes		XIIb0009-X0010CTAg3
VIN	29-Oct-08	816	7.31	2.74 x 10 ⁻⁶	6658	1:5	Negative	Yes		XIIb0009-X0010CTAg1
VIN	04-Nov-08	1744	4.15	3.68 x 10 ⁻⁶	4818	1:5	Negative	Yes		XIIb0009-X0010CTAg2
VIN	06-Nov-08	3641	6.20	2.50 x 10 ⁻⁶	5057	1:5	Negative	Yes		XIIb0009-X0010CTAg3

Abbreviations: ATZ = Atrazine; BBP = Butyl benzyl phthalate; DDT = *o,p'*-DDT; DMSO = Dimethyl sulfoxide; EC₅₀ = half-maximal effective concentration; EE = Ethinyl estradiol; E2 = 17β-estradiol; FLA = Flavone; GEN = Genistein; I.D. = Identification; MET = Methoxychlor; NON = *p*-n-nonylphenol; VIN = Vinclozolin

¹ 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 IIb Agonist Comprehensive Test Plates Tested at ECVAM

Test Substance	Date	DMSO	Induction ¹	E2 Reference Standard EC ₅₀ (µg/mL)	MET	Test Substance Dilution	Test Substance EC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
ATZ	12-Nov-08	3805	4.84	5.26x10 ⁻⁷	6894	1:2	2.63 x 10 ⁰	Yes		Ag CT1 V0005 V0006
ATZ	27-Nov-08	6100	3.22	2.32 x 10 ⁻⁶	7905	1:2	Positive but EC ₅₀ could not be calculated	Yes		Ag CT2 V0005 V0006
ATZ	01-Dec-08	14386	1.71	3.13 x 10 ⁻⁶	6425	1:2	Negative	No	Failed DMSO and Induction	Ag CT3 V0005 V0006
ATZ	13-Dec-08	3872	5.61	1.75 x 10 ⁻⁶	8453	1:2	2.91 x 10 ⁰	Yes		Ag CT4 V0005 V0006
BBP	16-Nov-08	3735	4.71	2.78 x 10 ⁻⁶	7245	1:2	7.86 x 10 ⁻¹	Yes		Ag CT1 V0011 V0012
BBP	27-Nov-08	6209	3.12	2.06 x 10 ⁻⁶	8322	1:2	3.29 x 10 ⁻¹	Yes		Ag CT2 V0011 V0012
BBP	01-Dec-08	4973	5.81	5.0 x 10 ⁻⁶	7832	1:2	9.20 x 10 ⁻¹	Yes		Ag CT3 V0011 V0012
DDT	16-Nov-08	3256	5.51	3.43 x 10 ⁻⁶	6875	1:5	1.59 x 10 ⁻¹	Yes		Ag CT1 V0009 V0010
DDT	27-Nov-08	4260	4.32	1.06 x 10 ⁻⁶	8941	1:5	1.65 x 10 ⁻¹	Yes		Ag CT2 V0009 V00010
DDT	01-Dec-08	8411	3.25	1.85 x 10 ⁻⁶	8673	1:5	1.33 x 10 ⁻¹	No	Failed DMSO	Ag CT3 V0009 V0010
DDT	13-Dec-08	4260	0.72	Negative	- 1275 87	1:5	Negative	No	Experimenter error.	Ag CT4 V0009 V0010
DDT	17-Dec-08	3535	3.86	1.04 x 10 ⁻⁶	8566	1:5	1.25 x 10 ⁻¹	Yes		Ag CT6 V0009 V0010
EE	16-Nov-08	3735	4.71	2.78 x 10 ⁻⁶	7245	1:5	2.04 x 10 ⁻⁶	Yes		Ag C1 V0011 V0012
EE	27-Nov-08	6209	3.12	2.06 x 10 ⁻⁶	8322	1:5	1.25 x 10 ⁻⁶	Yes		Ag CT2 V0011 V0012
EE	01-Dec-08	4973	5.81	5.0 x 10 ⁻⁶	7832	1:5	1.91 x 10 ⁻⁶	Yes		Ag CT3 V0011 V0012
FLA	16-Nov-08	3640	4.40	3.29 x 10 ⁻⁶	8260	1:2	1.75 x 10 ⁰	Yes		Ag CT1 V0007 V0008
FLA	27-Nov-08	7980	2.59	2.69 x 10 ⁻⁶	9220	1:2	9.23 x 10 ⁻²	No	Failed DMSO and Induction	Ag CT2 V0007 V0008
FLA	01-Dec-08	11137	2.47	1.78 x 10 ⁻⁶	7819	1:2	Negative	No	Failed DMSO and Induction	Ag CT3 V0007 V0008
FLA	13-Dec-08	4609	4.33	1.48 x 10 ⁻⁶	8823	1:2	1.36 x 10 ⁰	Yes		Ag CT4 V0007 V0008
FLA	17-Dec-08	3743	5.22	8.29 x 10 ⁻⁷	9285	1:2	1.59 x 10 ⁰	Yes		Ag CT5 V0007 V0008
GEN	16-Nov-08	3640	4.40	3.29 x 10 ⁻⁶	8260	1:5	8.33 x 10 ⁻²	Yes		Ag CT1 V0007 V0008

Test Substance	Date	DMSO	Induction ¹	E2 Reference Standard EC ₅₀ (µg/mL)	MET	Test Substance Dilution	Test Substance EC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
GEN	27-Nov-08	7980	2.59	2.69 x 10 ⁻⁶	9220	1:5	Negative	No	Failed DMSO and Induction	Ag CT2 V0007 V0008
GEN	01-Dec-08	11137	2.47	1.78 x 10 ⁻⁶	7819	1:5	10.82 x 10 ⁻²	No	Failed DMSO and Induction	Ag CT3 V0007 V0008
GEN	13-Dec-08	4609	4.33	1.48 x 10 ⁻⁶	8823	1:2	8.85 x 10 ⁻²	Yes		Ag CT4 V0007 V0008
GEN	17-Dec-08	3743	5.22	8.29 x 10 ⁻⁷	9285	1:2	7.14 x 10 ⁻²	Yes		Ag CT5 V0007V0008
NON	16-Nov-08	3256	5.51	3.43 x 10 ⁻⁶	6875	1:2	7.81 x 10 ⁻¹	Yes		Ag CT1 V0009 V0010
NON	27-Nov-08	4260	4.32	1.06 x 10 ⁻⁶	8941	1:2	3.13 x 10 ⁻¹	Yes		Ag CT2 V0009 V00010
NON	01-Dec-08	8411	3.25	1.85 x 10 ⁻⁶	8673	1:2	7.22 x 10 ⁻¹	No	Failed DMSO	Ag CT3 V0009 V0010
NON	13-Dec-08	4260	0.72	Negative	- 1275 87	1:5	Negative	No	Experimenter error.	Ag CT4 V0009 V0010
NON	17-Dec-08	3535	3.86	1.04 x 10 ⁻⁶	8566	1:5	5.57 x 10 ⁻¹	Yes		Ag CT6 V0009 V0010
VIN	12-Nov-08	3805	4.84	5.26x10 ⁻⁷	6894	1:2	5.03x10 ⁻¹	Yes		Ag CT1 V0005 V0006
VIN	27-Nov-08	6100	3.22	2.32 x 10 ⁻⁶	7905	1:2	24.30 x 10 ⁻¹	Yes		Ag CT2 V0005 V0006
VIN	01-Dec-08	14386	1.71	3.13 x 10 ⁻⁶	6425	1:2	Positive but EC ₅₀ could not be calculated	No	Failed DMSO and Induction	Ag CT3 V0005 V0006
VIN	13-Dec-08	3872	5.61	1.75 x 10 ⁻⁶	8453	1:2	8.86 x 10 ⁻¹	Yes		Ag CT4 V0005 V0006

Abbreviations: ATZ = Atrazine; BBP = Butyl benzyl phthalate; DDT = *o,p'*-DDT; DMSO = Dimethyl sulfoxide; EC₅₀ = half-maximal effective concentration; EE = Ethinyl estradiol; E2 = 17β-estradiol; FLA = Flavone; GEN = Genistein; I.D. = Identification; MET = Methoxychlor; NON = *p*-n-nonylphenol; VIN =- Vinclozolin

¹ 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 IIb Agonist Comprehensive Test Plates Tested at Hiyoshi

Test Substance	Date	DMSO	Induction ¹	E2 Reference Standard EC ₅₀ (µg/mL)	MET	Test Substance Dilution	Test Substance EC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
ATZ	07-Nov-08	7922	2.27	2.07 x 10 ⁻⁶	6706	1:2	Negative	No	Failed Induction	AgCTT081107-Ag4-1(fail)
ATZ	21-Nov-08	5735	4.32	3.75 x 10 ⁻⁶	8839	1:2	Positive but an EC ₅₀ could not be calculated	Yes		AgCTT081121-Ag4-2
ATZ	04-Dec-08	8078	3.27	2.31 x 10 ⁻⁶	9121	1:2	Negative	Yes		AgCTT081204-Ag4-3
ATZ	25-Dec-08	7009	3.44	3.09 x 10 ⁻⁶	7291	1:2	Negative	Yes		AgCTT081225-Ag4-4
BBP	07-Nov-08	6886	3.46	2.12 x 10 ⁻⁶	7139	1:5	8.30 x 10 ⁻¹	Yes		AgCTT081107-Ag1-1
BBP	21-Nov-08	5971	4.64	3.15 x 10 ⁻⁶	7212	1:5	9.93 x 10 ⁻¹	Yes		AgCTT081121-Ag1-2
BBP	04-Dec-08	7410	3.06	2.46 x 10 ⁻⁶	8949	1:5	Positive but an EC ₅₀ could not be calculated	Yes		AgCTT081204Ag1-3
DDT	07-Nov-08	6886	3.46	2.12 x 10 ⁻⁶	7139	1:5	2.70 x 10 ⁻¹	Yes		AgCTT081107-Ag1-1
DDT	21-Nov-08	5971	4.64	3.15 x 10 ⁻⁶	7212	1:5	2.62 x 10 ⁻¹	Yes		AgCTT081121-Ag1-2
DDT	04-Dec-08	7410	3.06	2.46 x 10 ⁻⁶	8949	1:5	2.10 x 10 ⁻¹	Yes		AgCTT081204-Ag1-3
EE	07-Nov-08	13265	1.61	2.43 x 10 ⁻⁶	5821	1:5	Positive but an EC ₅₀ could not be calculated	No	Failed DMSO and Induction	AgCTT081107-Ag2-1
EE	21-Nov-08	5761	4.64	2.73 x 10 ⁻⁶	6909	1:5	3.02 x 10 ⁻⁶	Yes		AgCTT081121-Ag2-2
EE	04-Dec-08	7897	3.06	1.79 x 10 ⁻⁶	9229	1:5	1.85 x 10 ⁻⁶	Yes		AgCTT081204-Ag2-3
EE	19-Dec-08	4955	4.86	3.09 x 10 ⁻⁶	7838	1:5	2.58 x 10 ⁻⁶	Yes		AgCTT081219-Ag2-4
FLA	07-Nov-08	13265	1.61	2.43 x 10 ⁻⁶	5821	1:5	Negative	No	Failed DMSO and Induction	AgCTT081107-Ag2-1
FLA	21-Nov-08	5761	4.64	2.73 x 10 ⁻⁶	6909	1:5	Positive but an EC ₅₀ could not be calculated	Yes		AgCTT081121-Ag2-2
FLA	04-Dec-08	7897	3.06	1.79 x 10 ⁻⁶	9229	1:5	Positive but an EC ₅₀ could not be calculated	Yes		AgCTT081204-Ag2-3

Test Substance	Date	DMSO	Induction ¹	E2 Reference Standard EC50 (µg/mL)	MET	Test Substance Dilution	Test Substance EC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
FLA	19-Dec-08	4955	4.86	3.09 x 10 ⁻⁶	7838	1:5	Positive but an EC ₅₀ could not be calculated	Yes		AgCTT081219-Ag2-4
GEN	07-Nov-08	4182	4.43	3.23 x 10 ⁻⁶	7482	1:5	5.14 x 10 ⁻²	Yes		AgCTT081107-Ag3-1
GEN	21-Nov-08	5942	4.62	2.49 x 10 ⁻⁶	7176	1:5	12.13 x 10 ⁻²	Yes		AgCTT081121-Ag3-2
GEN	04-Dec-08	8836	3.04	2.60 x 10 ⁻⁶	1040 9	1:5	14.10 x 10 ⁻²	No	Failed DMSO Acceptance	AgCTT081204-AG3-3(fail)
GEN	19-Dec-08	5053	4.82	3.02 x 10 ⁻⁶	7265	1:5	16.10 x 10 ⁻²	Yes		AgCTT081219-Ag3-4
GEN	25-Dec-08	6665	3.48	2.72 x 10 ⁻⁶	7748	1:5	14.03 x 10 ⁻²	Yes		AgCTT081225-Ag3-5
NON	07-Nov-08	7922	2.27	2.07 x 10 ⁻⁶	6706	1:2	Negative	No	Failed Induction	AgCTT081107-Ag4-1(fail)
NON	21-Nov-08	5735	4.32	3.75 x 10 ⁻⁶	8839	1:2	1.33 x 10 ⁰	Yes		AgCTT081121-Ag4-2
NON	04-Dec-08	8078	3.27	2.31 x 10 ⁻⁶	9121	1:2	1.24 x 10 ⁰	Yes		AgCTT081204-Ag4-3
NON	25-Dec-08	7009	3.44	3.09 x 10 ⁻⁶	7291	1:2	Positive but an EC ₅₀ could not be calculated	Yes		AgCTT081225-Ag4-4
VIN	07-Nov-08	4182	4.43	3.23 x 10 ⁻⁶	7482	1:2	Negative	Yes		AgCTT081107-Ag3-1
VIN	21-Nov-08	5942	4.62	2.49 x 10 ⁻⁶	7176	1:2	Positive but an EC ₅₀ could not be calculated	Yes		AgCTT081121-Ag3-2
VIN	04-Dec-08	8836	3.04	2.60 x 10 ⁻⁶	1040 9	1:2	Positive but an EC ₅₀ could not be calculated	No	Failed DMSO Acceptance	AgCTT081204-AG3-3(fail)
VIN	19-Dec-08	5053	4.82	3.02 x 10 ⁻⁶	7265	1:2	Negative	Yes		AgCTT081219-Ag3-4
VIN	25-Dec-08	6665	3.48	2.72 x 10 ⁻⁶	7748	1:2	Negative	Yes		AgCTT081225-Ag3-5

Abbreviations: ATZ = Atrazine; BBP = Butyl benzyl phthalate; DDT = *o,p'*-DDT; DMSO = Dimethyl sulfoxide; EC₅₀ = half-maximal effective concentration; EE = Ethinyl estradiol; E2 = 17β-estradiol; FLA = Flavone; GEN = Genistein; I.D. = Identification; MET = Methoxychlor; NON = *p*-n-nonylphenol; VIN = Vinclozolin

¹ 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 IIb Antagonist Plates Tested at XDS

Test Substance	Date	DMSO	Reduction ¹	Ral/E2 Reference Standard IC ₅₀ (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC ₅₀ (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
API	16-Nov-08	1733	16.36	6.28 x 10 ⁻⁴	7154	1608	1:2	Negative	Yes		XIIbX0023-X0024CTAnt1
API	18-Nov-08	930	8.77	4.56 x 10 ⁻⁴	9047	4631	1:2	Negative	Yes		XIIbX0023-X0024CTAnt2
API	19-Nov-08	1749	14.43	5.60 x 10 ⁻⁴	8042	2891	1:2	Negative	Yes		XIIbX0023-X0024CTAnt3
ATZ	16-Nov-08	1733	16.36	6.28 x 10 ⁻⁴	7154	1608	1:2	Negative	Yes		XIIbX0023-X0024CTAnt1
ATZ	18-Nov-08	930	8.77	4.56 x 10 ⁻⁴	9047	4631	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XIIbX0023-X0024CTAnt2
ATZ	19-Nov-08	1749	14.43	5.60 x 10 ⁻⁴	8042	2891	1:2	Negative	Yes		XIIbX0023-X0024CTAnt3
BBP	14-Nov-08	2635	12.50	5.23 x 10 ⁻⁴	8147	3314	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XIIbX0021-X0022CTAnt1
BBP	16-Nov-08	1542	11.88	6.32 x 10 ⁻⁴	8364	2180	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XIIbX0021-X0022CTAnt2
BBP	18-Nov-08	964	9.18	3.37 x 10 ⁻⁴	9795	5993	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XIIbX0021-X0022CTAnt3
CORT	14-Nov-08	2635	12.50	5.23 x 10 ⁻⁴	8147	3314	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XIIbX0021-X0022CTAnt1
CORT	16-Nov-08	1542	11.88	6.32 x 10 ⁻⁴	8364	2180	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XIIbX0021-X0022CTAnt2
CORT	18-Nov-08	964	9.18	3.37 x 10 ⁻⁴	9795	5993	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XIIbX0021-X0022CTAnt3
DDT	13-Nov-08	2714	8.88	4.41 x 10 ⁻⁴	7701	3349	1:2	Negative	Yes		XIIbX0017-X0018CTAnt1

Test Substance	Date	DMSO	Reduction ¹	Ral/E2 Reference Standard IC ₅₀ (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC ₅₀ (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used	Experiment I.D.
DDT	14-Nov-08	1639	8.90	5.50 x 10 ⁻⁴	7826	3132	1:2	Negative	Yes		XIIbX0017-X0018CTAnt2
DDT	16-Nov-08	1781	10.57	6.69 x 10 ⁻⁴	7814	1601	1:2	Negative	Yes		XIIbX0017-X0018CTAnt3
FLA	13-Nov-08	2773	11.92	5.53 x 10 ⁻⁴	8316	3635	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XIIbX0019-X0020CTAnt1
FLA	14-Nov-08	1941	10.29	5.51 x 10 ⁻⁴	8955	3598	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XIIbX0019-X0020CTAnt2
FLA	16-Nov-08	2516	13.16	6.34 x 10 ⁻⁴	7945	2042	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XIIbX0019-X0020CTAnt3
GEN	13-Nov-08	2773	11.92	5.53 x 10 ⁻⁴	8316	3635	1:2	Negative	Yes		XIIbX0019-X0020CTAnt1
GEN	14-Nov-08	1941	10.29	5.51 x 10 ⁻⁴	8955	3598	1:2	Negative	Yes		XIIbX0019-X0020CTAnt2
GEN	16-Nov-08	2516	13.16	6.34 x 10 ⁻⁴	7945	2042	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XIIbX0019-X0020CTAnt3
RES	13-Nov-08	2714	8.88	4.41 x 10 ⁻⁴	7701	3349	1:2	Negative	Yes		XIIbX0017-X0018CTAnt1
RES	14-Nov-08	1639	8.90	5.50 x 10 ⁻⁴	7826	3132	1:2	Negative	Yes		XIIbX0017-X0018CTAnt2
RES	16-Nov-08	1781	10.57	6.69 x 10 ⁻⁴	7814	1601	1:2	Negative	Yes		XIIbX0017-X0018CTAnt3

Abbreviations: API = Apigenin; ATZ = Atrazine; BBP = Butyl benzyl phthalate; Cont = Control; CORT = Corticosterone; DDT = *o,p'*-DDT; DMSO = Dimethyl sulfoxide; E2 = 17β-estradiol; Fla = Flavone; GEN = Genistein; IC₅₀ = half-maximal inhibitory concentration; I.D. = Identification; Ral = Raloxifene HCl; RES = Resveratrol

¹ 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 IIb Antagonist Plates Tested at ECVAM

Test Substance	Date	DMSO	Reduction ¹	Ral/E2 Reference Standard IC ₅₀ (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
API	20-Nov-08	3675	7.51	1.44 x 10 ⁻³	8597	1199	1:2	Negative	No	Ral\E2 Curve not Sigmoidal	Anta CT1 V0089 V0090
API	05-Dec-08	10088	6.40	6.42 x 10 ⁻⁴	10047	-1607	1:2	Negative	No	Failed DMSO Acceptance	Anta CT2 V0089 V0090
API	09-Dec-08	4134	6.31	7.88 x 10 ⁻⁴	9141	1264	1:2	Positive but an IC ₅₀ could not be calculated	Yes		Anta CT3 V0089 V0090
API	13-Dec-08	4147	5.37	6.18 x 10 ⁻⁴	10707	1600	1:2	Negative	Yes		Anta CT4 V0089 V0090
API	17-Dec-08	5449	6.40	6.16 x 10 ⁻⁴	7938	4s84	1:2	Positive but an IC ₅₀ could not be calculated	Yes		Anta CT5 V0089 V0090
ATZ	20-Nov-08	3118	7.72	1.37 x 10 ⁻³	8818	860	1:2	Positive but an IC ₅₀ could not be calculated	No	Ral\E2 Curve not Sigmoidal	Anta CT1 V0087 V0088
ATZ	05-Dec-08	9946	5.81	4.23 x 10 ⁻⁴	7025	-635	1:2	Negative	No	Failed DMSO Acceptance	Anta CT2 V0087 V0088
ATZ	09-Dec-08	3212	7.72	5.86 x 10 ⁻⁴	9221	1075	1:2	Positive but an IC ₅₀ could not be calculated	Yes		Anta CT3 V0087 V0088
ATZ	13-Dec-08	4427	5.87	6.03 x 10 ⁻⁴	9053	1577	1:2	Positive but an IC ₅₀ could not be calculated	Yes		Anta CT4 V0087 V0088
ATZ	17-Dec-08	5053	6.90	6.46 x 10 ⁻⁴	8435	419	1:2	Positive but an IC ₅₀ could not be calculated	Yes		Anta CT5 V0087 V0088
BBP	20-Nov-08	2522	6.07	2.93 x 10 ⁻⁴	10399	1321	1:2	Negative	Yes		Anta CT1 V0085 V0086

Test Substance	Date	DMSO	Reduction ¹	Ral/E2 Reference Standard IC ₅₀ (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
BBP	05-Dec-08	14111	5.41	4.70 x 10 ⁻⁴	11625	-8081	1:2	Positive but an IC ₅₀ could not be calculated	No	Failed DMSO Acceptance	Anta CT2 V0085 V0086
BBP	09-Dec-08	3217	7.05	5.92 x 10 ⁻⁴	9226	755	1:2	Positive but an IC ₅₀ could not be calculated	Yes		Anta CT3 V0085 V0086
BBP	13-Dec-08	5577	6.40	6.00 x 10 ⁻⁴	10010	603	1:2	Negative	Yes		Anta CT4 V0085 V0086
CORT	20-Nov-08	2522	6.07	2.93 x 10 ⁻⁴	10399	1321	1:2	Positive but an IC ₅₀ could not be calculated	Yes		Anta CT1 V0085 V0086
CORT	05-Dec-08	14111	5.41	4.70 x 10 ⁻⁴	11625	-8081	1:2	4.79 x 10 ⁺¹	No	Failed DMSO Acceptance	Anta CT2 V0085 V0086
CORT	09-Dec-08	3217	7.05	5.92 x 10 ⁻⁴	9226	755	1:2	3.98 x 10 ⁺¹	Yes		Anta CT3 V0085 V0086
CORT	13-Dec-08	5577	6.40	6.00 x 10 ⁻⁴	10010	603	1:2	5.15 x 10 ⁺¹	Yes		Anta CT4 V0085 V0086
DDT	20-Nov-08	2654	7.16	3.74 x 10 ⁻⁴	10319	694	1:2	Positive but an IC ₅₀ could not be calculated	Yes		Anta CT1 V0083 V0084
DDT	05-Dec-08	14948	5.89	4.37 x 10 ⁻⁴	9685	-16714	1:2	Negative	No	Failed DMSO Acceptance	Anta CT2 V0083 V0084
DDT	09-Dec-08	5398	7.02	6.80 x 10 ⁻⁴	9454	-286	1:2	Negative	Yes		Anta CT3 V0083 V0084
DDT	13-Dec-08	4794	6.58	5.59 x 10 ⁻⁴	8373	185	1:2	Positive but an IC ₅₀ could not be calculated	Yes		Anta CT4 V0083 V0084
FLA	20-Nov-08	3675	7.51	1.44 x 10 ⁻³	8597	1199	1:2	Positive but an IC ₅₀ could not be calculated	No	Ral\E2 Curve not Sigmoidal	Anta CT1 V0089 V0090
FLA	05-Dec-08	10088	6.40	6.42 x 10 ⁻⁴	10047	-1607	1:2	Positive but an IC ₅₀ could not be calculated	No	Failed DMSO Acceptance	Anta CT2 V0089 V0090

Test Substance	Date	DMSO	Reduction ¹	Ral/E2 Reference Standard IC ₅₀ (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
FLA	09-Dec-08	4134	6.31	7.88 x 10 ⁻⁴	9141	1264	1:2	Positive but an IC ₅₀ could not be calculated	Yes		Anta CT3 V0089 V0090
FLA	13-Dec-08	4147	5.37	6.18 x 10 ⁻⁴	10707	1600	1:2	Positive but an IC ₅₀ could not be calculated	Yes		Anta CT4 V0089 V0090
FLA	17-Dec-08	5449	6.40	6.16 x 10 ⁻⁴	7938	484	1:2	Positive but an IC ₅₀ could not be calculated	Yes		Anta CT5 V0089 V0090
GEN	20-Nov-08	2654	7.16	3.74 x 10 ⁻⁴	10319	694	1:2	Positive but an IC ₅₀ could not be calculated	Yes		Anta CT1 V0083 V0084
GEN	05-Dec-08	14948	5.89	4.37 x 10 ⁻⁴	9685	-16714	1:2	Positive but an IC ₅₀ could not be calculated	No	Failed DMSO Acceptance	Anta CT2 V0083 V0084
GEN	09-Dec-08	5398	7.02	6.80 x 10 ⁻⁴	9454	-286	1:2	Positive but an IC ₅₀ could not be calculated	Yes		Anta CT3 V0083 V0084
GEN	13-Dec-08	4794	6.58	5.59 x 10 ⁻⁴	8373	185	1:2	Positive but an IC ₅₀ could not be calculated	Yes		Anta CT4 V0083 V0084
RES	20-Nov-08	3118	7.72	1.37 x 10 ⁻³	8818	860	1:2	1.42 x 10 ¹	No	Ral\E2 Curve not Sigmoidal	Anta CT1 V0087 V0088
RES	05-Dec-08	9946	5.81	4.23 x 10 ⁻⁴	7025	-635	1:2	1.26 x 10 ¹	No	Failed DMSO Acceptance	Anta CT2 V0087 V0088
RES	09-Dec-08	3212	7.72	5.86 x 10 ⁻⁴	9221	1075	1:2	1.37 x 10 ¹	Yes		Anta CT3 V0087 V0088
RES	13-Dec-08	4427	5.87	6.03 x 10 ⁻⁴	9053	1577	1:2	1.70 x 10 ¹	Yes		Anta CT4 V0087 V0088
RES	17-Dec-08	5053	6.90	6.46 x 10 ⁻⁴	8435	419	1:2	1.53 x 10 ¹	Yes		Anta CT5 V0087 V0088

Abbreviations: API = Apigenin; ATZ = Atrazine; BBP = Butyl benzyl phthalate; Cont = Control; CORT = Corticosterone; DDT = *o,p'*-DDT; DMSO = Dimethyl sulfoxide; E2 = 17β-estradiol; Fla = Flavone; GEN = Genistein; IC₅₀ = half-maximal inhibitory concentration; I.D. = Identification; Ral = Raloxifene HCl; RES = Resveratrol

¹ 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 IIb Antagonist Plates Tested at Hiyooshi

Test Substance	Date	DMSO	Reduction ¹	Ral/E2 Reference Standard IC ₅₀ (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
API	14-Nov-08	6047	8.81	7.42 x 10 ⁻⁴	5575	99	1:2	Negative	Yes		AntCTT081114-Ant2-1
API	29-Nov-08	7139	11.27	7.80 x 10 ⁻⁴	5105	4	1:2	Positive but IC ₅₀ could not be calculated	Yes		AntCTT081129-Ant2-2
API	12-Dec-08	5814	11.13	6.58 x 10 ⁻⁴	5790	174	1:2	Positive but IC ₅₀ could not be calculated	Yes		AntCTT081212-Ant2-3
API	19-Dec-08	5602	9.41	6.83 x 10 ⁻⁴	6018	344	1:2	Positive but IC ₅₀ could not be calculated	Yes		AntCTT081219-Ant2-4
ATZ	14-Nov-08	5231	12.90	7.24 x 10 ⁻⁴	5379	22	1:2	Positive but IC ₅₀ could not be calculated	Yes		AntCTT081114-Ant4-1
ATZ	29-Nov-08	6421	11.60	7.92 x 10 ⁻⁴	4569	189	1:2	Positive but IC ₅₀ could not be calculated	Yes		AntCTT081129-Ant4-2
ATZ	12-Dec-08	7053	7.92	6.97 x 10 ⁻⁴	5361	90	1:2	Positive but IC ₅₀ could not be calculated	Yes		AntCTT081212-Ant4-3
BBP	14-Nov-08	6338	9.82	7.43 x 10 ⁻⁴	5135	-26	1:2	Positive but IC ₅₀ could not be calculated	Yes		AntCTT081114-Ant1-1
BBP	29-Nov-08	7789	8.79	7.80 x 10 ⁻⁴	5939	-336	1:2	Negative	Yes		AntCTT081129-Ant1-2
BBP	12-Dec-08	5143	11.37	7.20 x 10 ⁻⁴	5181	223	1:2	Negative	Yes		AntCTT081212-Ant1-3
BBP	19-Dec-08	5250	9.58	6.65 x 10 ⁻⁴	5409	422	1:2	Positive but IC ₅₀ could not be calculated	Yes		AntCTT081219-Ant1-4
CORT	14-Nov-08	4633	14.31	7.21 x 10 ⁻⁴	4603	30	1:2	Positive but IC ₅₀ could not be calculated	Yes		AntCTT081114-Ant3-1

Test Substance	Date	DMSO	Reduction ¹	Ral/E2 Reference Standard IC ₅₀ (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
CORT	29-Nov-08	7336	11.15	9.78 x 10 ⁻⁴	4522	94	1:2	Positive but IC ₅₀ could not be calculated	Yes		AntCTT081129-Ant3-2
CORT	12-Dec-08	7992	11.46	7.13 x 10 ⁻⁴	5197	-113	1:2	Positive but IC ₅₀ could not be calculated	Yes		AntCTT081212-Ant3-3
DDT	14-Nov-08	6338	9.82	7.43 x 10 ⁻⁴	5135	-26	1:2	Positive but IC ₅₀ could not be calculated	Yes		AntCTT081114-Ant1-1
DDT	29-Nov-08	7789	8.79	7.80 x 10 ⁻⁴	5939	-336	1:2	Negative	Yes		AntCTT081129-Ant1-2
DDT	12-Dec-08	5143	11.37	7.20 x 10 ⁻⁴	5181	223	1:2	Negative	Yes		AntCTT081212-Ant1-3
DDT	19-Dec-08	5250	9.58	6.65 x 10 ⁻⁴	5409	422	1:2	Positive but IC ₅₀ could not be calculated	Yes		AntCTT081219-Ant1-4
FLA	14-Nov-08	6047	8.81	7.42 x 10 ⁻⁴	5575	99	1:2	Negative	Yes		AntCTT081114-Ant2-1
FLA	29-Nov-08	7139	11.27	7.80 x 10 ⁻⁴	5105	4	1:2	Negative	Yes		AntCTT081129-Ant2-2
FLA	12-Dec-08	5814	11.13	6.58 x 10 ⁻⁴	5790	174	1:2	Negative	Yes		AntCTT081212-Ant2-3
FLA	19-Dec-08	5602	9.41	6.83 x 10 ⁻⁴	6018	344	1:2	Negative	Yes		AntCTT081219-Ant2-4
GEN	14-Nov-08	5231	12.90	7.24 x 10 ⁻⁴	5379	22	1:2	Negative	Yes		AntCTT081114-Ant4-1
GEN	29-Nov-08	6421	11.60	7.92 x 10 ⁻⁴	4569	189	1:2	Negative	Yes		AntCTT081129-Ant4-2
GEN	12-Dec-08	7053	7.92	6.97 x 10 ⁻⁴	5361	90	1:2	Negative	Yes		AntCTT081212-Ant4-3
RES	14-Nov-08	4633	14.31	7.21 x 10 ⁻⁴	4603	30	1:2	2.49 x 10 ¹	Yes		AntCTT081114-Ant3-1
RES	29-Nov-08	7336	11.15	9.78 x 10 ⁻⁴	4522	94	1:2	2.32 x 10 ¹	Yes		AntCTT081129-Ant3-2

Test Substance	Date	DMSO	Reduction ¹	Ral/E2 Reference Standard IC ₅₀ (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
RES	12-Dec-08	7992	11.46	7.13 x 10 ⁻⁴	5197	-113	1:2	2.60 x 10 ¹	Yes		AntCTT081212-Ant3-3

Abbreviations: API = Apigenin; ATZ = Atrazine; BB = Butyl benzyl phthalate; Cont = Control; CORT = Corticosterone; DDT = *o,p'*-DDT; DMSO = Dimethyl sulfoxide; E2 = 17β-estradiol; Fla = Flavone; GEN = Genistein; IC₅₀ = half-maximal inhibitory concentration; I.D. = Identification; Ral = Raloxifene HCl; RES = Resveratrol

¹ 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.

Appendix K4-1

**Phase 2b Repeat Testing and XDS New Personnel
Proficiency Testing**

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Table 13 Phase IIb Repeat Testing at ECVAM - Agonist Comprehensive Test Plates

Test Substance	Date	DMSO	Induction ¹	E2 Reference Standard EC ₅₀ (µg/mL)	MET	Test Substance Dilution	Test Substance EC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
ATZ	20-May-09	1917	14.46	1.83 x 10 ⁻⁶	5798	1:5	Positive, but an EC ₅₀ could not be calculated	Yes		Ag CT1 V0027 V2001
ATZ	27-May-09	2599	10.77	1.68 x 10 ⁻⁶	7592	1:5	5.65 x 10 ⁻¹	Yes		Ag CT2 V2001 CT1 V0045
ATZ	10-Jun-09	3340	8.29	2.94 x 10 ⁻⁶	8196	1:5	Positive, but an EC ₅₀ could not be calculated	Yes		Ag CT3 V2001 E2 control old DMSO
ATZ	27-Jun-09	2382	12.20	1.31 x 10 ⁻⁶	7166	1:5	2.06 x 10 ⁰	Yes		Ag CT1 V2001 V2002 NEW
ATZ	30-Sep-09	1795	11.71	5.54 x 10 ⁻⁷	6698	1:5	Positive, but an EC ₅₀ could not be calculated	Yes		Ag CT2 V2001 V2002 NEW
ATZ	07-Oct-09	32761	0.97	Positive, but an EC ₅₀ could not be calculated	11761	1:5	Positive, but an EC ₅₀ could not be calculated	No	Failed DMSO Control, Induction, E2 Reference Standard	Ag CT3 V2001 V2002 NEW
ATZ	14-Oct-09	3196	7.72	3.15 x 10 ⁻⁶	8076	1:5	Positive, but an EC ₅₀ could not be calculated	Yes		Ag CT4 V2001 V2002 NEW
VIN	27-Jun-09	2382	12.20	1.31 x 10 ⁻⁶	7166	1:2	Positive, but an EC ₅₀ could not be calculated	Yes		Ag CT1 V2001 V2002 NEW
VIN	30-Sep-09	1795	11.71	5.54 x 10 ⁻⁷	6698	1:2	Positive, but an EC ₅₀ could not be calculated	Yes		Ag CT2 V2001 V2002 NEW
VIN	07-Oct-09	32761	0.97	Positive, but an EC ₅₀ could not be calculated	11761	1:2	Negative	No	Failed DMSO Control, Induction, E2 Reference Standard	Ag CT3 V2001 V2002 NEW

Test Substance	Date	DMSO	Induction ¹	E2 Reference Standard EC ₅₀ (µg/mL)	MET	Test Substance Dilution	Test Substance EC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
VIN	14-Oct-09	3196	7.72	3.15 x 10 ⁻⁶	8076	1:2	Negative	Yes		Ag CT4 V2001 V2002 NEW

Table 14 Phase IIb Repeat Testing at Hiyoshi – Antagonist Comprehensive Test Plates

Test Substance	Date	DMSO	Reduction ¹	Ral/E2 Reference Standard IC ₅₀ (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
FLA	01-Apr-09	6047	8.81	7.42 x 10 ⁻⁴	5575	99	1:2	Negative	Yes ²	Used wrong concentrations of FLA	AntCTT081101-AntAdd-1
FLA	23-Apr-09	3730	11.34	4.93 x 10 ⁻⁴	5660	387	1:2	Positive, but an IC ₅₀ could not be calculated	Yes		AntCTT081114-AntAdd-2
FLA	23-Apr-09	9806	10.40	1.30 x 10 ⁻³	3767	1094	1:2	Positive but an IC ₅₀ value could not be calculated	No	Failed DMSO Control	AntCTT081114-AntAdd-3
FLA	12-May-09	12103	8.62	6.11 x 10 ⁻⁴	4695	377	1:2	Positive but an IC ₅₀ value could not be calculated	No	Failed DMSO Control	AntCTT081114-AntAdd-4
FLA	15-May-09	8857	7.27	7.13 x 10 ⁻⁴	4935	1011	1:2	Positive but an IC ₅₀ value could not be calculated	No	Failed DMSO Control	AntCTT081114-AntAdd-5
FLA	22-May-09	4248	10.15	5.50 x 10 ⁻⁴	5363	1293	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		AntCTT081114-AntAdd-6
FLA	29-May-09	4983	9.49	6.43 x 10 ⁻⁴	5247	1187	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		AntCTT081101-AntAdd-7
GEN	01-Apr-09	6047	8.81	7.42 x 10 ⁻⁴	5575	99	1:2	Negative	Yes ²	Used wrong concentrations of GEN	AntCTT081101-AntAdd-1
GEN	23-Apr-09	3730	11.34	4.93 x 10 ⁻⁴	5660	387	1:2	2.56 x 10 ¹	Yes		AntCTT081114-AntAdd-2

Test Substance	Date	DMSO	Reduction ¹	Ral/E2 Reference Standard IC ₅₀ (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
GEN	23-Apr-09	9806	10.40	1.30 x 10 ⁻³	3767	1094	1:2	Positive but an IC ₅₀ value could not be calculated	No	Failed DMSO Control	AntCTT081114-AntAdd-3
GEN	12-May-09	12103	8.62	6.11 x 10 ⁻⁴	4695	377	1:2	Positive but an IC ₅₀ value could not be calculated	No	Failed DMSO Control	AntCTT081114-AntAdd-4
GEN	15-May-09	8857	7.27	7.13 x 10 ⁻⁴	4935	1011	1:2	Positive but an IC ₅₀ value could not be calculated	No	Failed DMSO Control	AntCTT081114-AntAdd-5
GEN	22-May-09	4248	10.15	5.50 x 10 ⁻⁴	5363	1293	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		AntCTT081114-AntAdd-6
GEN	29-May-09	4983	9.49	6.43 x 10 ⁻⁴	5247	1187	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		AntCTT081101-AntAdd-7

Abbreviations: Cont = Control; DMSO = Dimethyl sulfoxide; E2 = 17β-estradiol; Fla = Flavone; GEN = Genistein; IC₅₀ = half-maximal inhibitory concentration; I.D. = Identification; Ral = Raloxifene HCl

¹ 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.

² Abnormal test substance response during this experiment led to the shipment of replacement coded test substance to Hiyoshi

Table 15 New Experimenter Proficiency Testing at XDS – Agonist Range Finder

Experiment I.D.	Date	Induction ¹	DMSO	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used
lumi agonist 1 27 2009	27-Jan-09	3.33	1618	Yes	

¹ 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 16 New Experimenter Proficiency Testing at XDS – Antagonist Range Finder

Experiment I.D.	Date	Reduction ¹	DMSO	Was Plate Used for Data Analysis ?	Reason Why Plate Was Not Used
lumi antagonist 1 27 2009	27 Jun 09	4.32	2295	Yes	

¹ 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 17 New Experimenter Proficiency Testing at XDS – Agonist Comprehensive Test Plates

Test Substance	Date	DMSO	Induction ¹	E2 Reference Standard EC ₅₀ (µg/mL)	MET	Test Substance Dilution	Test Substance EC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
ATZ	06-Mar-09	13029	1.09	Negative	35581	1:5	Negative	No	Failed DMSO, E2 Reference Standard, Induction	AgCT_X0010_X0015 030609
ATZ	31-Mar-09	1499	3.24	1.39 x 10 ⁻⁵	3413	1:5	5.28 x 10 ⁻²	No	Abnormal cell growth	AgCT_X0015_X009033109
ATZ	11-Apr-09	642	11.25	3.03 x 10 ⁻⁶	4437	1:5	4.32 x 10 ⁻⁵	Yes		Lumi agonist 4 11 2009_X0015_X0016
ATZ	01-May-09	572	5.40	3.14 x 10 ⁻⁶	7557	1:5	Positive but an EC ₅₀ value could not be calculated	Yes		XPTX0010X0023AGCT090501
ATZ	07-May-09	1049	3.89	4.22 x 10 ⁻⁶	5300	1:5	Positive but an EC ₅₀ value could not be calculated	Yes		XPTX0023AGCT090507
BPA	06-Mar-09	12823	1.22	Positive but an EC ₅₀ value could not be calculated	-6995	1:5	Negative	No	Failed DMSO, Induction, and MET	AgCT_X0094_X009030609
BPA	16-Apr-09	1152	3.17	2.28 x 10 ⁻⁶	2809	1:5	4.11 x 10 ⁻²	Yes		XPTX0085X0094AGCT090416
BPA	30-Apr-09	7755	1.82	1.83 x 10 ⁻⁶	3314	1:5	Positive but an EC ₅₀ value could not be calculated	No	Failed Induction	XPTX0094X0085AGCT090430
BPA	01-May-09	647	5.02	3.50 x 10 ⁻⁶	5214	1:5	7.23 x 10 ⁻²	Yes		XPTX0085X0094AGCT090501
DES	06-Mar-09	13510	1.02	Negative	-74511	1:5	Negative	No	Failed DMSO, Induction, and MET	AgCT_X0085_X0016 030609

Test Substance	Date	DMSO	Induction ¹	E2 Reference Standard EC ₅₀ (µg/mL)	MET	Test Substance Dilution	Test Substance EC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
DES	16-Apr-09	1152	3.17	2.28 x 10 ⁻⁶	2809	1:5	6.34 x 10 ⁻⁷	Yes		XPTX0085X0094AG CT090416
DES	30-Apr-09	7755	1.82	1.83 x 10 ⁻⁶	3314	1:5	2.86 x 10 ⁻⁶	No	Failed Induction	XPTX0094X0085AG CT090430
DES	01-May-09	647	5.02	3.50 x 10 ⁻⁶	5214	1:5	2.59 x 10 ⁻⁵	Yes		XPTX0085X0094AG CT090501
DES	07-May-09	2962	3.18	3.75 x 10 ⁻⁶	4650	1:5	1.66 x 10 ⁻⁵	Yes		XPTX0085X0010AG CT090507
EE	06-Mar-09	13510	1.02	Negative	-74511	1:5	Negative	No	Failed DMSO, Induction, and MET	AgCT_X0085_X001 6 030609
EE	11-Apr-09	642	11.25	3.03 x 10 ⁻⁶	4437	1:5	2.71 x 10 ⁻⁶	Yes		Lumi agonist 4 11 2009_X0015_X0016
EE	18-Apr-08	1228	3.00	7.20 x 10 ⁻⁷	5288	1:5	Positive but an EC ₅₀ value could not be calculated	No	Failed Induction	Lumi agonist 4 18 2009_X0010_X0016
EE	07-May-09	2584	3.35	3.35 x 10 ⁻⁶	5973	1:5	1.25 x 10 ⁻⁶	Yes		XPTX0016X0010AG CT090507
NON	01-May-09	572	5.40	3.14 x 10 ⁻⁶	7557	1:5	Positive but an EC ₅₀ value could not be	Yes		XPTX0010X0023AG CT090501
NON	06-Mar-09	13029	1.09	Negative	35581	1:5	Positive but an EC ₅₀ value could not be calculated	No	Failed DMSO, E2 Reference Standard, Induction	AgCT_X0010_X001 5 030609
NON	11-Mar-09	206	9.07	2.68 x 10 ⁻⁶	5216	1:5	Positive but an EC ₅₀ value could not be calculated		Yes	AgCT_X0009_X001 0 031109
NON	18-Apr-08	1228	3.00	7.20 x 10 ⁻⁷	5288	1:5	Positive but an EC ₅₀ value could not be calculated	No	Failed Induction	Lumi agonist 4 18 2009_X0010_X0016

Test Substance	Date	DMSO	Induction ¹	E2 Reference Standard EC ₅₀ (µg/mL)	MET	Test Substance Dilution	Test Substance EC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
NON	07-May-09	2584	3.35	3.35 x 10 ⁻⁶	5973	1:5	Positive but an EC ₅₀ value could not be calculated	Yes		XPTX0016X0010AG CT090507
NON	07-May-09	2962	3.18	3.75 x 10 ⁻⁶	4650	1:5	Positive but an EC ₅₀ value could not be calculated	Yes		XPTX0085X0010AG CT090507
VIN	06-Mar-09	12823	1.22	Positive but an EC ₅₀ value could not be calculated	-6995	1:5	Negative	No	Failed DMSO, Induction, and MET	AgCT_X0094_X009 030609
VIN	11-Mar-09	206	9.07	2.68 x 10 ⁻⁶	5216	1:5	Positive but an EC ₅₀ value could not be calculated		Yes	AgCT_X0009_X001 0 031109
VIN	31-Mar-09	1499	3.24	1.39 x 10 ⁻⁵	3413	1:5	Positive but an EC ₅₀ value could not be calculated	No	Abnormal cell growth	AgCT_X0015_X009 033109

Abbreviations: ATZ = Atrazine; BPA = Bisphenol A; DES = Diethylstilbestrol; DMSO = Dimethyl sulfoxide; EC₅₀ = half maximal effective concentration; E2 = 17β-estradiol; EE = 17α-ethinyl estradiol; I.D. = Identification; NON = Nonylphenol; Vin = Vinclozolin

¹ 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 18 New Experimenter Proficiency Testing (and Phase IIb Retests) at XDS – Antagonist Comprehensive Test Plates

Test Substance	Date	DMSO	Reduction ¹	Ral/E2 Reference Standard IC ₅₀ (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
API	03-May-09	3253	6.98	7.94 x 10 ⁻⁴	6447	2705	1:2	2.56 x 10 ¹	Yes		XPTX0011X0054ANTCT090503_1
API	03-May-09	3133	4.27	7.34 x 10 ⁻⁴	6995	6096	1:2	2.48 x 10 ¹	Yes		XPTX0011X0054ANTCT090503_2
API	07-May-09	3224	8.10	7.20 x 10 ⁻⁴	6575	2768	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XPTX0011X0054ANTCT090507
ATZ	07-May-09	2489	6.10	5.94 x 10 ⁻⁴	7893	2799	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XPTX0023X0017ANTCT090507
ATZ	22-May-09	1943	6.39	7.45 x 10 ⁻⁴	8366	5097	1:2	Negative	Yes		XPTX0019X0023CT090522
ATZ	22-May-09	5231	9.37	8.75 x 10 ⁻⁴	5811	-836	1:2	Negative	Yes		XPTX0019X0023CT090522_2
ATZ	09-Jun-09	912	3.83	1.16 x 10 ⁻³	-3995	237690	1:2	Negative	No	Failed E2, FLA\E2	XPTX0019X0023ANTCT090609
ATZ	26-Jun-09	1245	6.06	7.70 x 10 ⁻⁴	9355	3563	1:2	Negative	No	Failed E2. FLA\E2	XPTX0019X0023ANTCT090630
ATZ	24-Nov-09	5009	5.18	4.64 x 10 ⁻⁴	8575	1410	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		2009-11-24XPIIIAntCTX0082X0023
ATZ	05-Nov-09	926	13.02	3.13 x 10 ⁻⁴	8387	3465	1:2	Negative	Yes	Experimenter error	2009-11-4-XPIIIANTCTX0023X0065

Test Substance	Date	DMSO	Reduction ¹	Ral/E2 Reference Standard IC ₅₀ (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
CORT	30-Apr-09	11983	7.92	1.12 x 10 ⁻³	6558	-4711	1:2	Negative	No	Failed E2. FLA\E2	XPTX0020X0021ANTCT090430
CORT	01-May-09	661	5.38	7.49 x 10 ⁻⁴	6792	3784	1:2	Negative	Yes		XPTX0020X0021ANTCT090501
CORT	03-May-09	2935	4.56	6.96 x 10 ⁻⁴	7333	4273	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XPTX0020X0021ANTCT090503
CORT	07-May-09	2718	4.91	7.39 x 10 ⁻⁴	6888	3036	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XPTX0021X0048ANTCT090507
DBA	03-May-09	3772	4.64	6.86 x 10 ⁻⁴	8862	3120	1:5	Negative	No	Failed FLA\E2	XPTX0048X0026ANTCT090503_1
DBA	03-May-09	3987	4.21	6.35 x 10 ⁻⁴	6286	3591	1:5	Positive but an IC ₅₀ value could not be calculated	Yes		XPTX0048X0026ANTCT090503_2
DBA	07-May-09	2718	4.91	7.39 x 10 ⁻⁴	6888	3036	1:5	Positive but an IC ₅₀ value could not be calculated	Yes		XPTX0021X0048ANTCT090507
DDT	03-May-09	3253	6.98	7.94 x 10 ⁻⁴	6447	2705	1:5	Positive but an IC ₅₀ value could not be calculated	Yes		XPTX0011X0054ANTCT090503_1
DDT	03-May-09	3133	4.27	7.34 x 10 ⁻⁴	6995	6096	1:5	Positive but an IC ₅₀ value could not be calculated	Yes		XPTX0011X0054ANTCT090503_2

Test Substance	Date	DMSO	Reduction ¹	Ral/E2 Reference Standard IC ₅₀ (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
DDT	07-May-09	3224	8.10	7.20 x 10 ⁻⁴	6575	2768	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XPTX0011X0054ANTCT090507
FLA	30-Apr-09	11983	7.92	1.12 x 10 ⁻³	6558	-4711	1:2	Positive but an IC ₅₀ value could not be calculated	No	Failed DMSO, Fla\E2	XPTX0020X0021ANTCT090430
FLA	01-May-09	661	5.38	7.49 x 10 ⁻⁴	6792	3784	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XPTX0020X0021ANTCT090501
FLA	03-May-09	2935	4.56	6.96 x 10 ⁻⁴	7333	4273	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XPTX0020X0021ANTCT090503
GEN	07-May-09	2765	6.54	5.93 x 10 ⁻⁴	5567	2618	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XPTX0017X0019ANTCT090507
GEN	22-May-09	1943	6.39	7.45 x 10 ⁻⁴	8366	5097	1:2	Negative	Yes		XPTX0019X0023CT090522
GEN	22-May-09	5231	9.37	8.75 x 10 ⁻⁴	5811	-836	1:2	Negative	Yes		XPTX0019X0023CT090522_2
GEN	09-Jun-09	912	3.83	1.16 x 10 ⁻³	-3995	237690	1:2	Negative	No	Failed E2, Fla\E2	XPTX0019X0023ANTCT090609
GEN	26-Jun-09	1245	6.06	7.70 x 10 ⁻⁴	10829	3563	1:2	Negative	No	Failed E2 Control	XPTX0019X0023ANTCT090630
RES	07-May-09	2489	6.10	5.94 x 10 ⁻⁴	7893	2799	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XPTX0023X0017ANTCT090507

Test Substance	Date	DMSO	Reduction ¹	Ral/E2 Reference Standard IC ₅₀ (µg/mL)	E2 Cont	Fla\E2 Cont	Test Substance Dilution	Test Substance IC ₅₀ (µg/mL)	Was Plate Used for Data Analysis?	Reason Why Plate Was Not Used	Experiment I.D.
RES	07-May-09	2765	6.54	5.93 x 10 ⁻⁴	5567	2618	1:2	2.54 x 10 ¹	Yes		XPTX0017X0 019ANTCT09 0507
RES	19-May-09	1283	10.63	6.42 x 10 ⁻⁴	8350	2899	1:2	Positive but an IC ₅₀ value could not be calculated	Yes		XPTX0026X0 017ANTCT09 0519
TAM	03-May-09	3772	4.64	6.86 x 10 ⁻⁴	8862	3120	1:2	Positive but an IC ₅₀ value could not be calculated	No	Failed FLA\E2	XPTX0048X0 026ANTCT09 0503_1
TAM	03-May-09	3987	4.21	6.35 x 10 ⁻⁴	6286	3591	1:2	1.83 x 10 ⁻¹	Yes		XPTX0048X0 026ANTCT09 0503_2
TAM	19-May-09	1283	10.63	6.42 x 10 ⁻⁴	8350	2899	1:2	2.85 x 10 ⁻¹	Yes		XPTX0026X0 017ANTCT09 0519

Abbreviations: API = Apigenin; ATZ = Atrazine; Cont = Control; CORT = Corticosterone; DBA = Dibenzo [*a,h*]anthracene; DDT = *o,p'*-DDT; DMSO = Dimethyl sulfoxide; E2 = 17β-estradiol; Fla = Flavone; GEN = Genistein; IC₅₀ = half-maximal inhibitory concentration; I.D. = Identification; Ral = Raloxifene HCl; RES = Resveratrol; TAM = Tamoxifen

¹ 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.