

Appendix A

Phase I Experiments Conducted to Establish Historical Databases

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Table A-1 Phase I Agonist Plates Tested at XDS

Plate I.D.	Date	Induction ¹	EC ₅₀ (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used
XICT1BPA	01-Nov-07	3.7	2.8 x 10 ⁻⁶	Yes	
XICT2BPA	01-Nov-07	4.9	1.9 x 10 ⁻⁶	Yes	
XICT3BPA	02-Nov-07	4.1	2.0 x 10 ⁻⁶	Yes	
XICT4BPA	02-Nov-07	5.3	5.8 x 10 ⁻⁶	Yes	
XICT5BPA	02-Nov-07	5.3	2.7 x 10 ⁻⁶	Yes	
XICT6BPA	02-Nov-07	5.8	2.4 x 10 ⁻⁶	Yes	
XICT7BPA	02-Nov-07	3.9	1.7 x 10 ⁻⁶	Yes	
XICT8BPA	02-Nov-07	4.1	2.1 x 10 ⁻⁶	Yes	
XICT9BPA	08-Aug-07	4.7	2.2 x 10 ⁻⁶	Yes	
XICT10BPA	08-Aug-07	4.9	3.1 x 10 ⁻⁶	Yes	

¹ Induction for historical database plates is measured by dividing the averaged highest E2 reference standard RLU value by the averaged DMSO control RLU value.

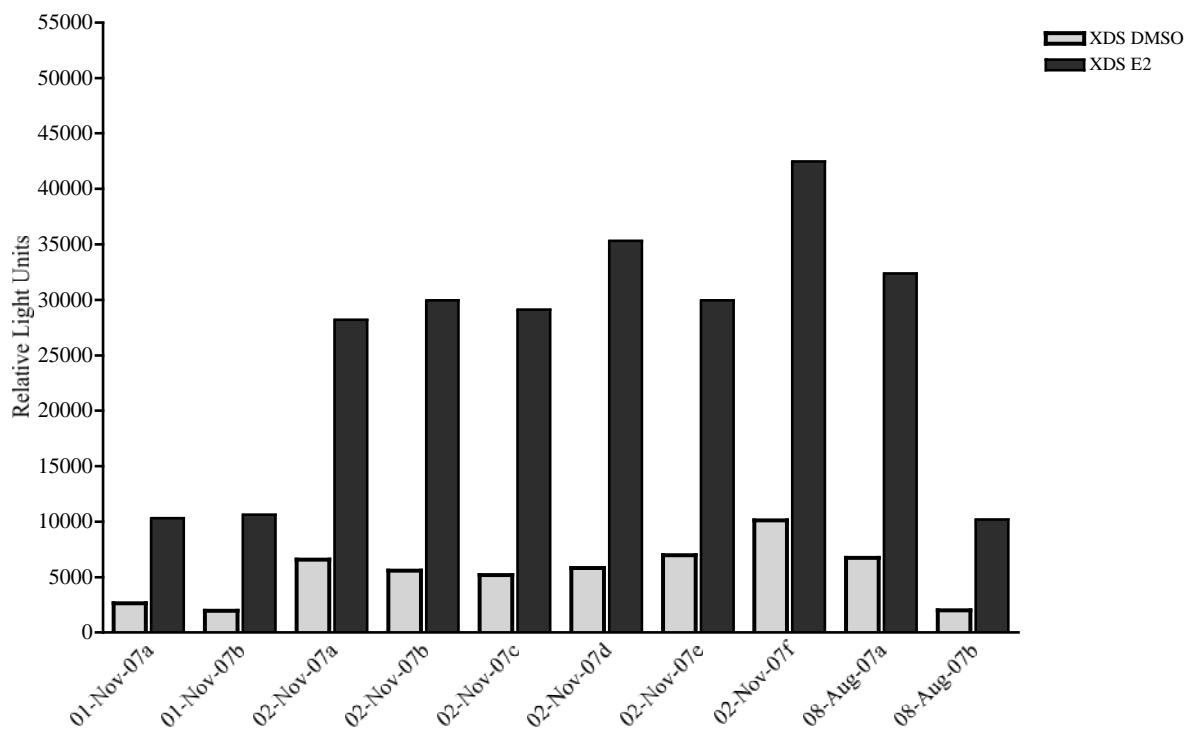
Figure A-1 Highest Non-Adjusted E2 and DMSO Values from Plates Tested at XDS

Table A-2 Phase I Agonist Plates Tested at ECVAM

Plate I.D.	Date	Induction ¹	EC ₅₀ (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used
Ag09112007-001	09-Nov-07	7.7	2.1 x 10 ⁻⁶	Yes	
Ag09112007-002	09-Nov-07	9.3	2.7 x 10 ⁻⁶	Yes	
Ag13112007-001	13-Nov-07	8.1	2.8 x 10 ⁻⁶	Yes	
Ag13112007-002	13-Nov-07	10.1	2.3 x 10 ⁻⁶	Yes	
Ag16112007-001	16-Nov-07	8.8	2.1 x 10 ⁻⁶	Yes	
Ag16112007-002	16-Nov-07	7.3	1.9 x 10 ⁻⁶	Yes	
Ag19112007-001	19-Nov-07	7.3	2.7 x 10 ⁻⁶	Yes	
Ag19112007-002	19-Nov-07	6.7	2.5 x 10 ⁻⁶	Yes	
Ag22112007-001	22-Nov-07	7.8	3.7 x 10 ⁻⁶	Yes	
Ag22112007-002	22-Nov-07	7.4	4.1 x 10 ⁻⁶	Yes	
Ag25112007-001	25-Nov-07	8.4	3.5 x 10 ⁻⁶	Yes	
Ag25112007-002	25-Nov-07	9.0	3.6 x 10 ⁻⁶	Yes	
Ag28112007-001	28-Nov-07	8.1	3.3 x 10 ⁻⁶	Yes	
Ag28112007-002	28-Nov-07	6.9	3.2 x 10 ⁻⁶	Yes	
Ag01122007-001	01-Dec-07	8.7	3.4 x 10 ⁻⁶	Yes	
Ag01122007-002	01-Dec-07	8.8	3.1 x 10 ⁻⁶	Yes	
Ag04122007-001	04-Dec-07	7.9	3.5 x 10 ⁻⁶	Yes	
Ag04122007-002	04-Dec-07	6.9	3.5 x 10 ⁻⁶	Yes	

¹ Induction for historical database plates is measured by dividing the averaged highest E2 reference standard RLU value by the averaged DMSO control RLU value.

Figure A-2 Highest Non-Adjusted E2 and DMSO Values from Plates Tested at ECVAM

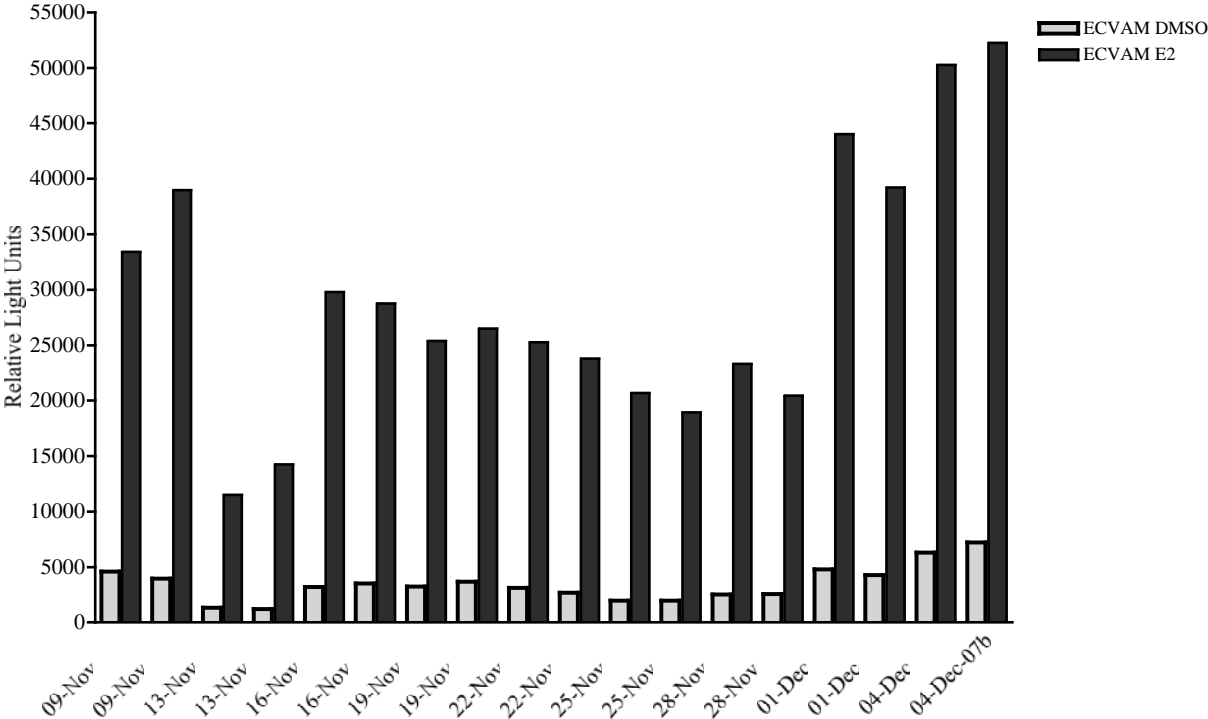


Table A-3 Phase I Agonist Plates Tested at Hiyoshi

Plate I.D.	Date	Induction ¹	EC ₅₀ (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used
HirefsubAg1	28-May-07	5.2	2.7 x 10 ⁻⁶	Yes	
HirefsubAg2	30-May-07	4.8	3.8 x 10 ⁻⁶	Yes	
HirefsubAg3	04-Jun-07	1.6	4.9 x 10 ⁻⁶	No	Failed Induction
HirefsubAg4	06-Jun-07	2.7	5.5 x 10 ⁻⁶	No	Failed Induction
HirefsubAg5	11-Jun-07	3.5	2.8 x 10 ⁻⁶	Yes	
HirefsubAg6	13-Jun-07	3.9	2.7 x 10 ⁻⁶	Yes	
HirefsubAg7	18-Jun-07	3.8	3.6 x 10 ⁻⁶	Yes	
HirefsubAg8	20-Jun-07	5.0	2.2 x 10 ⁻⁶	Yes	
HirefsubAg9	25-Jun-07	4.0	4.1 x 10 ⁻⁶	Yes	
HirefsubAg10	27-Jun-07	3.8	1.8 x 10 ⁻⁶	Yes	
HirefsubAg11	02-Jul-07	4.5	2.8 x 10 ⁻⁶	Yes	
HirefsubAg12	06-Jul-07	6.3	4.2 x 10 ⁻⁶	Yes	

¹ Induction for historical database plates is measured by dividing the averaged highest E2 reference standard RLU value by the averaged DMSO control RLU value.

Figure A-3 Highest Non-Adjusted E2 and DMSO Values from Plates Tested at Hiyoshi

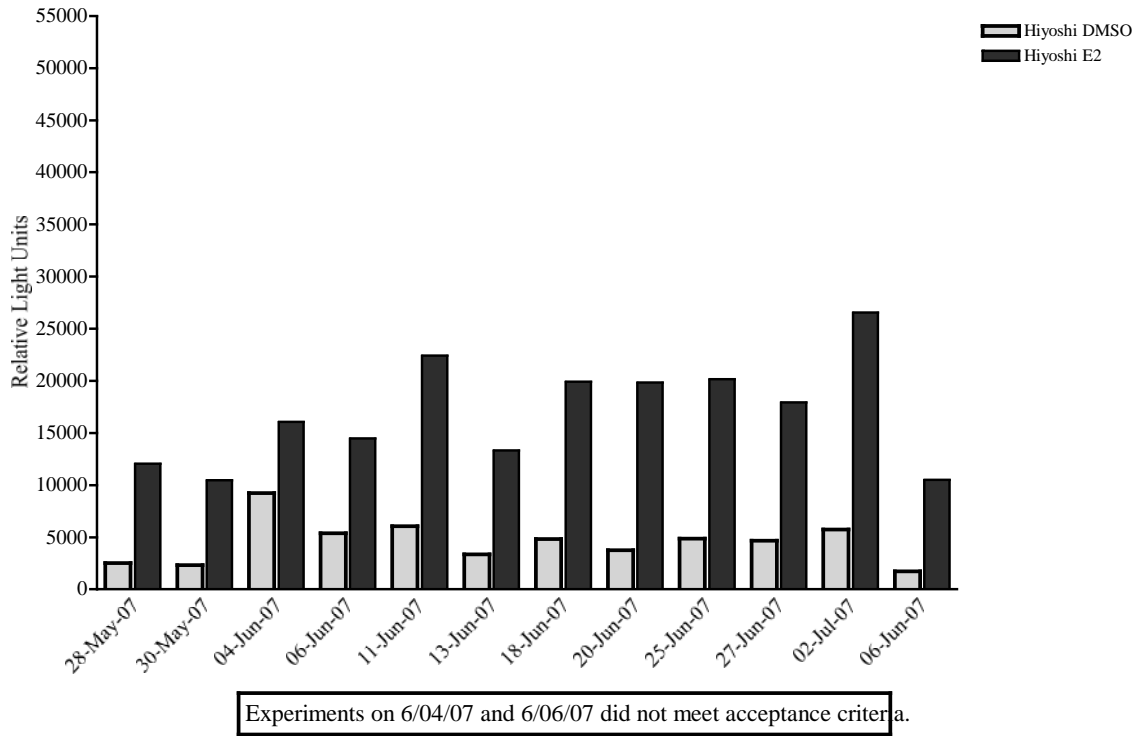
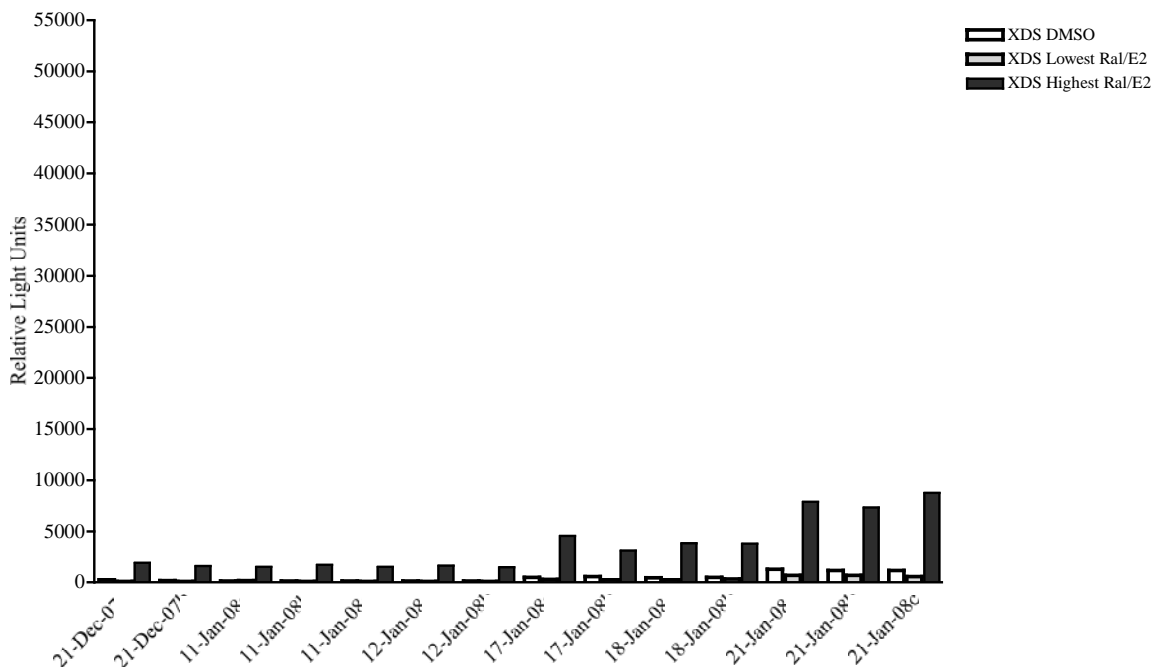


Table A-4 Phase I Antagonist Plates Tested at XDS

Experiment I.D.	Date	Reduction ¹	IC ₅₀ (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used
XICTAnt1.xls	21-Dec-07	19.62	4.65 x 10 ⁻⁴	Yes	
XICTAnt2.xls	21-Dec-07	15.98	5.18 x 10 ⁻⁴	Yes	
XICTAnt3.xls	22-Dec-07			No	Plate Contaminated
XICTAnt4.xls	11-Jan-08	14.01	4.07 x 10 ⁻⁴	Yes	
XICTAnt5.xls	11-Jan-08	15.230	4.43 x 10 ⁻⁴	Yes	
XICTAnt6.xls	11-Jan-08	13.08	3.89 x 10 ⁻⁴	Yes	
XICTAnt7.xls	12-Jan-08	14.33	2.87 x 10 ⁻⁴	Yes	
XICTAnt8.xls	12-Jan-08	14.61	4.07 x 10 ⁻⁴	Yes	
XICTAnt9.xls	17-Jan-08	15.46	3.67 x 10 ⁻⁴	Yes	
XICTAnt10.xls	17-Jan-08	12.07	3.33 x 10 ⁻⁴	Yes	
XICTAnt11.xls	18-Jan-08	15.20	4.29 x 10 ⁻⁴	Yes	
XICTAnt12.xls	18-Jan-08	12.67	2.91 x 10 ⁻⁴	Yes	
XICTAnt13.xls	21-Jan-08	11.85	5.71 x 10 ⁻⁴	Yes	
XICTAnt14.xls	21-Jan-08	9.43	4.86 x 10 ⁻⁴	Yes	
XICTAnt15.xls	21-Jan-08	15.13	5.65 x 10 ⁻⁴	Yes	

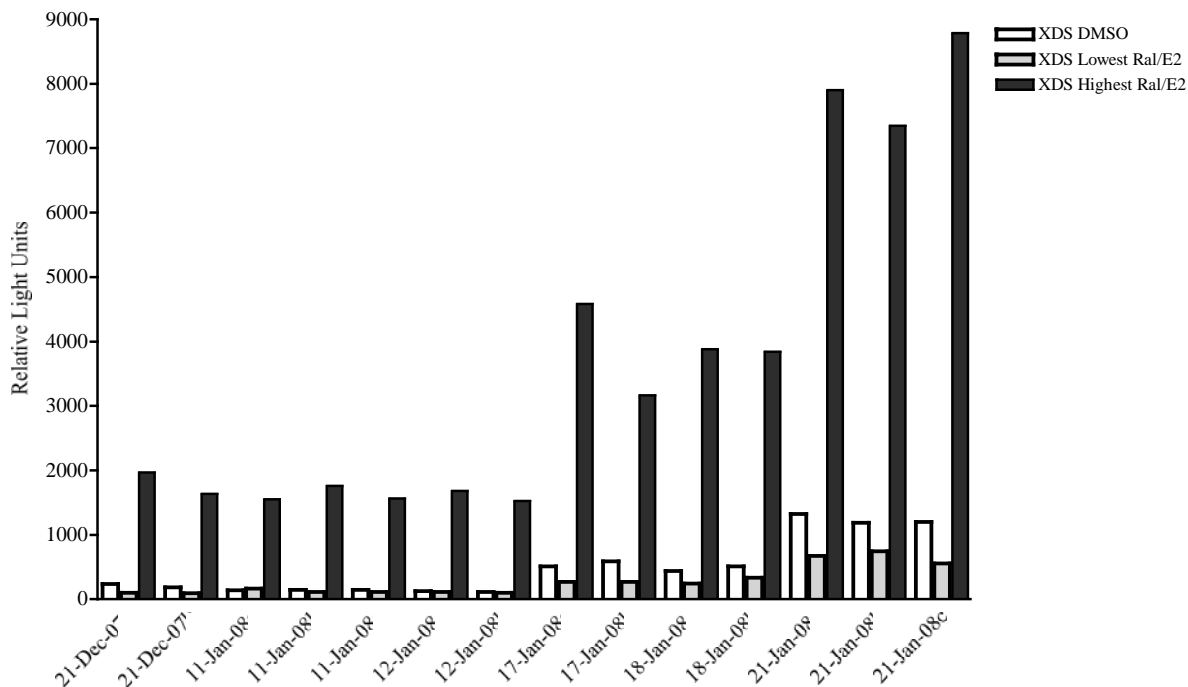
¹ Reduction for historical database plates is measured by dividing the averaged highest Ral/E2 reference standard RLU value by the lowest averaged Ral/E2 reference standard RLU value.

Figure A-4 Highest and Lowest Non-Adjusted Ral/E2 and DMSO Values from Plates Tested at XDS¹



¹ The y-axis in this figure is set to the same scale as that used for the ECVAM and Hiyoshi data.

Figure A-5 Highest and Lowest Non-Adjusted Ral/E2 and DMSO Values from Plates Tested at XDS¹



¹ The y-axis in this figure has been reduced to improve visualization of DMSO and Lowest Ral/E2 data.

Table A-5 Phase I Antagonist Plates Tested at ECVAM

Plate I.D.	Date	Reduction ¹	IC ₅₀ (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used
Ag09112007-001	09-Nov-07	7.5	6.1 x 10 ⁻⁴	Yes	
Ag09112007-002	09-Nov-07	8.9	4.4 x 10 ⁻⁴	Yes	
Ag13112007-001	13-Nov-07	7.9	4.0 x 10 ⁻⁴	Yes	
Ag13112007-002	13-Nov-07	8.3	4.4 x 10 ⁻⁴	Yes	
Ag16112007-001	16-Nov-07	6.7	5.7 x 10 ⁻⁴	Yes	
Ag16112007-002	16-Nov-07	8.1	4.2 x 10 ⁻⁴	Yes	
Ag19112007-001	19-Nov-07	7.4	5.5 x 10 ⁻⁴	Yes	
Ag19112007-002	19-Nov-07	6.7	4.8 x 10 ⁻⁴	Yes	
Ag22112007-001	22-Nov-07	8.1	4.3 x 10 ⁻⁴	Yes	
Ag22112007-002	22-Nov-07	7.8	4.2 x 10 ⁻⁴	Yes	
Ag25112007-001	25-Nov-07	8.7	4.0 x 10 ⁻⁴	Yes	
Ag25112007-002	25-Nov-07	9.2	3.8 x 10 ⁻⁴	Yes	
Ag28112007-001	28-Nov-07	8.3	3.5 x 10 ⁻⁴	Yes	
Ag28112007-002	28-Nov-07	8.9	3.8 x 10 ⁻⁴	Yes	
Ag01122007-001	01-Dec-07	7.7	3.6 x 10 ⁻⁴	Yes	
Ag01122007-002	01-Dec-07	7.8	3.1 x 10 ⁻⁴	Yes	
Ag04122007-001	04-Dec-07	7.6	3.9 x 10 ⁻⁴	Yes	
Ag04122007-002	04-Dec-07	7.5	4.0 x 10 ⁻⁴	Yes	

¹ 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.

Figure A-6 Highest and Lowest Non-Adjusted Ral/E2 Values from Plates Tested at ECVAM

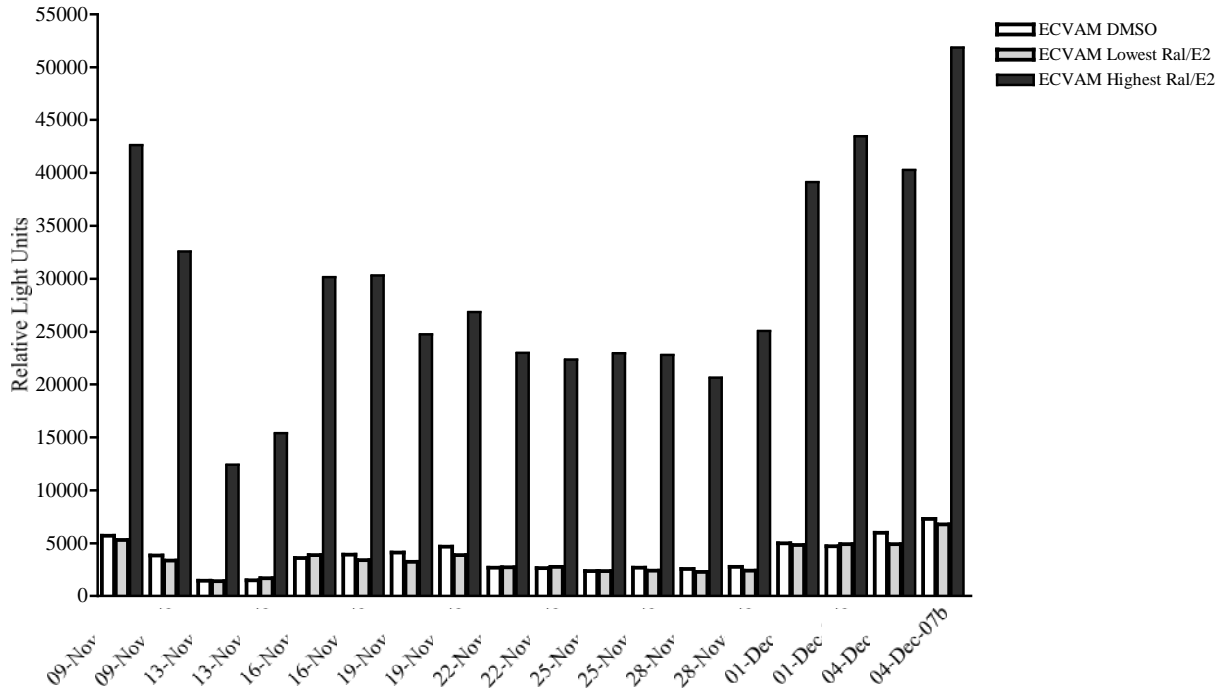
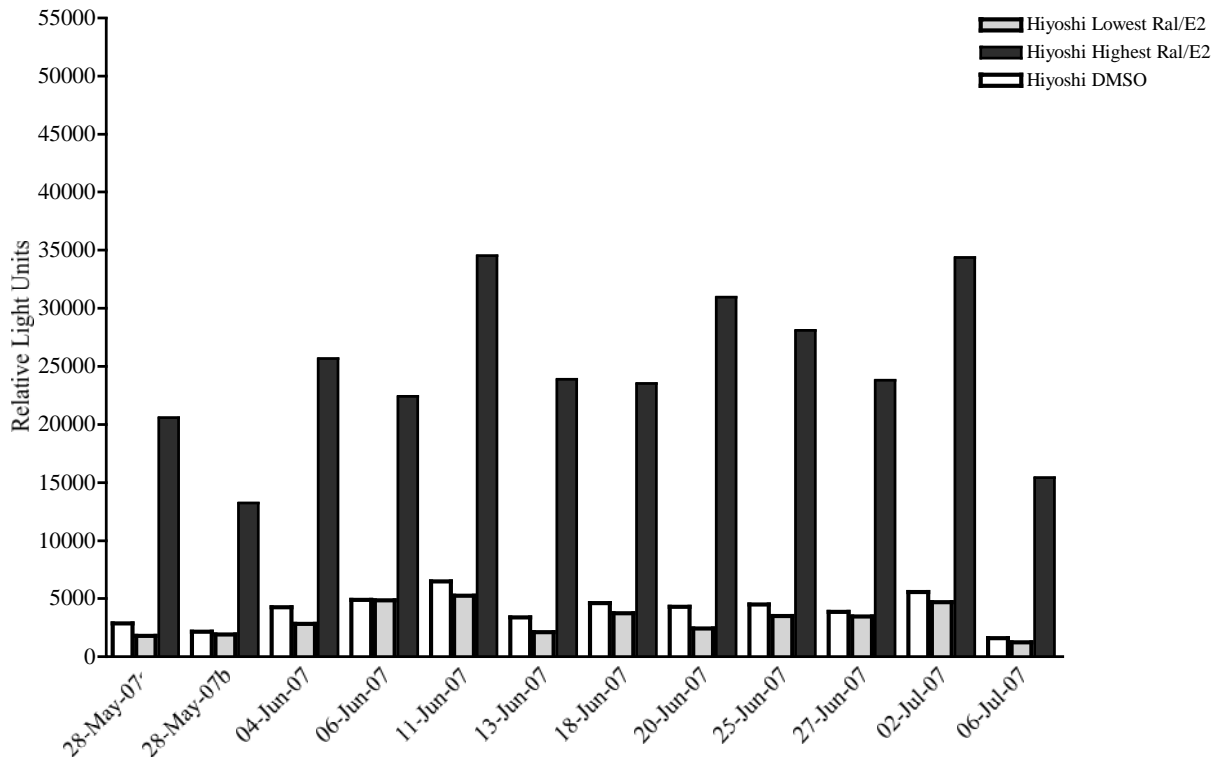


Table A-6 Phase I Antagonist Plates Tested at Hiyoshi

Experiment I.D.	Date	Reduction¹	IC₅₀ (µg/mL)	Was Plate Used for Data Analysis	Reason Why Plate Was Not Used
HirefsubAnt1	28-May-07	10.7	5.1 x 10 ⁻⁴	Yes	
HirefsubAnt2	28-May-07	6.6	5.9 x 10 ⁻⁴	Yes	
HirefsubAnt3	04-Jun-07	8.0	7.4 x 10 ⁻⁴	Yes	
HirefsubAnt4	06-Jun-07	4.3	6.2 x 10 ⁻⁴	Yes	
HirefsubAnt5	11-Jun-07	6.0	7.9 x 10 ⁻⁴	Yes	
HirefsubAnt6	13-Jun-07	10.0	5.4 x 10 ⁻⁴	Yes	
HirefsubAnt7	18-Jun-07	5.9	5.9 x 10 ⁻⁴	Yes	
HirefsubAnt8	20-Jun-07	11.4	5.5 x 10 ⁻⁴	Yes	
HirefsubAnt9	25-Jun-07	7.0	7.7 x 10 ⁻⁴	Yes	
HirefsubAnt10	27-Jun-07	6.6	7.7 x 10 ⁻⁴	Yes	
HirefsubAnt11	02-Jul-07	7.0	7.1 x 10 ⁻⁴	Yes	
HirefsubAnt12	06-Jul-07	11.0	3.7 x 10 ⁻⁴	Yes	

¹ 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.

Figure A-7 Highest and Lowest Non-Adjusted Ral/E2 Values from Plates Tested at Hiyoshi



References

ICCVAM. 2003. ICCVAM Evaluation of *In Vitro* Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays. NIH Pub. No. 03-4503. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: <http://iccvam.niehs.nih.gov/methods/endocrine.htm> [accessed 14 February 2006]

ICCVAM. 2006. ICCVAM Addendum to ICCVAM Evaluation of *In Vitro* Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays. NIH Pub. No. 03-4503 (Proposed Revisions to the List of Recommended Reference Substances for Validation). Research Triangle Park, NC: National Institute of Environmental Health Sciences Available: <http://iccvam.niehs.nih.gov/methods/endodocs/EDAddendum.pdf> [accessed 25 July 2006]

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