Methods

The objective of Task 6, WA 4-11 was to evaluate the inter-laboratory variability of IC_{50} and RBA values resulting from four laboratories conducting competitive binding assays using individual laboratory prepared "standard" cytosol and 10 test chemicals (Table 1). The saturation assays associated with these runs have already been analyzed in WA 4-11 Task 5 and Task 7 statistical reports, however the results of those analyses will be presented again for completeness. Lab A prepared the cytosol for Lab D, but did not participate in this task. Lab A's saturation assay results are presented for comparison. All other laboratories prepared their own cytosol.

The mean and coefficient of variation (CV) within laboratory results in measurement of K_d, number of receptors, and B_{max} from a saturation assay was evaluated to ensure that the laboratory was preparing the rat uterine cytosol correctly and could reliably measure the relevant descriptors. In addition, the variability in the competitive binding assay was calculated from the measurements of IC_{50} for R1881 and the weak binder (dexamethasone) and the relative binding affinity (RBA) for the weak binder and test chemicals. The goodness-of-fit (R^2 values ranging from 0 to 1) to the appropriate nonlinear binding equations were calculated.

Intra-laboratory variability of the resulting measurements was defined as the CV (standard deviation/mean x 100%) between separate assays run by the same laboratory (indicated by the date of the run). Inter-laboratory variability was defined as the CV between the mean laboratory statistics (average of the separate runs). Other sources of variability associated with the estimation process of these statistics include the non-specific binding goodness-of-fit to a simple linear model for both the saturation and competitive binding assays and the variability in the activity of the radioactive labels.

Observations were removed from data analysis by the submitting laboratory based on their determination of outliers and level of saturation. Observations were removed from the intra-laboratory comparison to allow convergence of the nonlinear one-site binding or competitive binding equations for the standard and weak positive only. The criteria used for model convergence and an appropriate measurement of the assay parameters were an R^2 value between 0 and 1, a $K_d > 0$, for the saturation assay. No observations other than those designated by the individual laboratories were removed from the test chemical runs.

Table 1. Test Chemicals Used in the Competitive Assay

Code Name	Chemical Name	CAS #	Initial Concentration
CR42400	5a-Androstan-17B-ol-3-one (4,5a-Dihydrotestosterone)	521-18-6	30.0 mM
CR42401	Testosterone	58-22-0	30.0 mM
CR42402	17β-Estradiol	50-28-2	30.0 mM
CR42403	6a-Methyl-17a-hydroxyprogesterone acetate (MPA)	71-58-9	30.0 mM
CR42404	Linuron	330-55-2	30.0 mM
CR42405	1,1-Dichloro-2,2-bis(4-chlorophenyl)ethane (pp'-DDE)	72-55-9	30.0 mM
CR42406	Cyproterone Acetate	427-51-0	30.0 mM
CR42407	Spironolactone	52-01-7	30.0 mM
CR42408	Bis(2-ethylhexyl) phthalate (DEPH)	117-81-7	30.0 mM
CR42409	Atrazine	1912-24-9	30.0 mM

Results

Saturation binding Assay: Three independent runs of the saturation assay with triplicate tubes of each concentration were conducted during earlier tasks using the cytosol that was also used in this task. The data used for the intra-laboratory comparison were presented in WA 4-11 Task 5 and Task 7 statistical reports. As part of this task, Lab D ran a single run of the saturation assay with triplicate tubes of each concentration using cytosol prepared by Lab A for Task 5. This run is presented in Appendix A.

The goodness-of-fit to the one-site binding equation ranged from 0.86 to 1.00 with a median value of 0.96 for the 16 runs (Table 2). The range of B_{max} (fmole/100 μg) values was 4.81 to 16.8 with a median value of 11.23. The range of K_d (nM) values was 0.66 to 3.77 with a median value of 0.92. The intra-laboratory CV for B_{max} ranged from 4.4% to 14% and for K_d from 2.9% to 23%. The variability in these measurements was relatively small. The difference in the saturation assay results from Lab A and Lab D using Lab A prepared cytosol is presented in Figure 1. The saturation assay results from all laboratories participating in Task 5 using their own preparation of cytosol is presented in Figure 2. Finally, the intra-laboratory variability between Task 5 and Task 7 preparation of cytosol from Lab E is presented in Figure 3.

Table 2. Intra-Laboratory variability of the statistics associated with the saturation assay.

Statistic	Assay	Lab A	Lab B	Lab C	Lab D	Lab E
Bmax (fmole/100 μg)	Task 5-1	6.24	5.30	14.3		14.4
Bmax (fmole/100 μg)	Task 5-2	6.10	4.81	16.8		12.3
Bmax (fmole/100 μg)	Task 5-3	5.72	5.32	15.3		15.6
Bmax (fmole/100 μg)	Task 6-1				6.96	
Bmax (fmole/100 μg)	Task 7-1					11.9
Bmax (fmole/100 μg)	Task 7-2					11.3
Bmax (fmole/100 μg)	Task 7-3					11.1
Kd (nM)	Task 5-1	1.92	0.699	0.835		0.930
Kd (nM)	Task 5-2	2.00	0.677	0.930		0.834
Kd (nM)	Task 5-3	2.88	0.660	1.03		0.926
Kd (nM)	Task 6-1				3.77	
Kd (nM)	Task 7-1					0.919
Kd (nM)	Task 7-2					0.904
Kd (nM)	Task 7-3					0.916
Goodness of Fit	Task 5-1	0.95	0.94	0.98		0.97
Goodness of Fit	Task 5-2	0.96	0.92	0.97		0.86
Goodness of Fit	Task 5-3	0.95	0.88	0.98		1.00
Goodness of Fit	Task 6-1				0.90	
Goodness of Fit	Task 7-1					0.97
Goodness of Fit	Task 7-2					0.97
Goodness of Fit	Task 7-3					0.94
		Lab A	**Lab B**	**Lab C**	**Lab D**	**Lab E**
Mean Bmax (fmole/100 μg)		6.02	5.14	15.4	6.96	12.8
CV Bmax		4.4%	5.6%	8.1%	NA	14%
Mean Kd (nM)		2.27	0.68	0.93	3.77	0.91
CV Kd		23.5%	2.90%	10.4%	NA	3.96%
Average Goodness of Fit		0.95	0.91	0.98	0.90	0.95

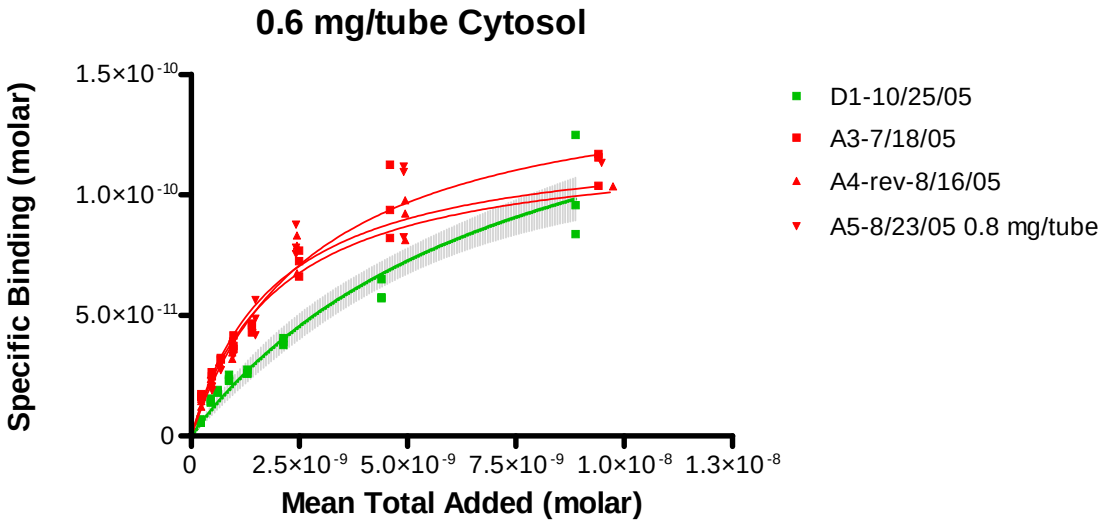

Figure 1. Inter-laboratory variability of one-site binding curves using Lab A prepared cytosol. The gray area represents the 95% confidence bands about the fitted curve.

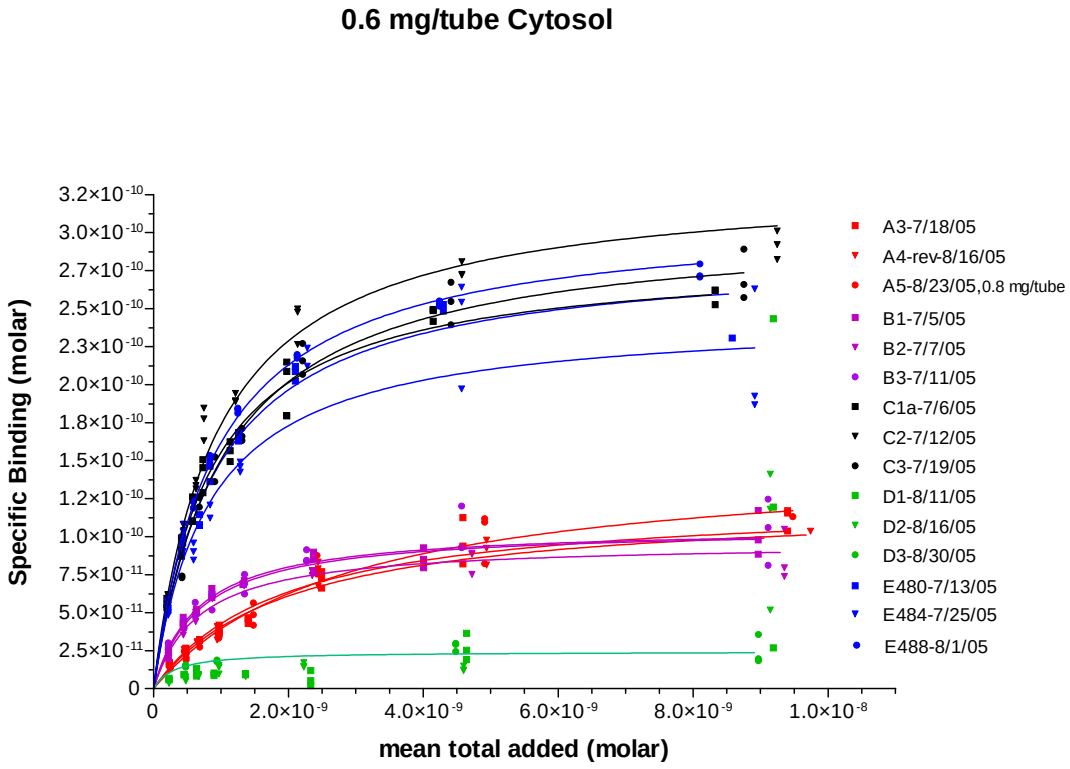

Figure 2. Inter-laboratory variability of one-site binding curves from Task 5 using individual laboratory prepared cytosol. The fitted curve for run C2-2/24/05 is without the designated outlier.

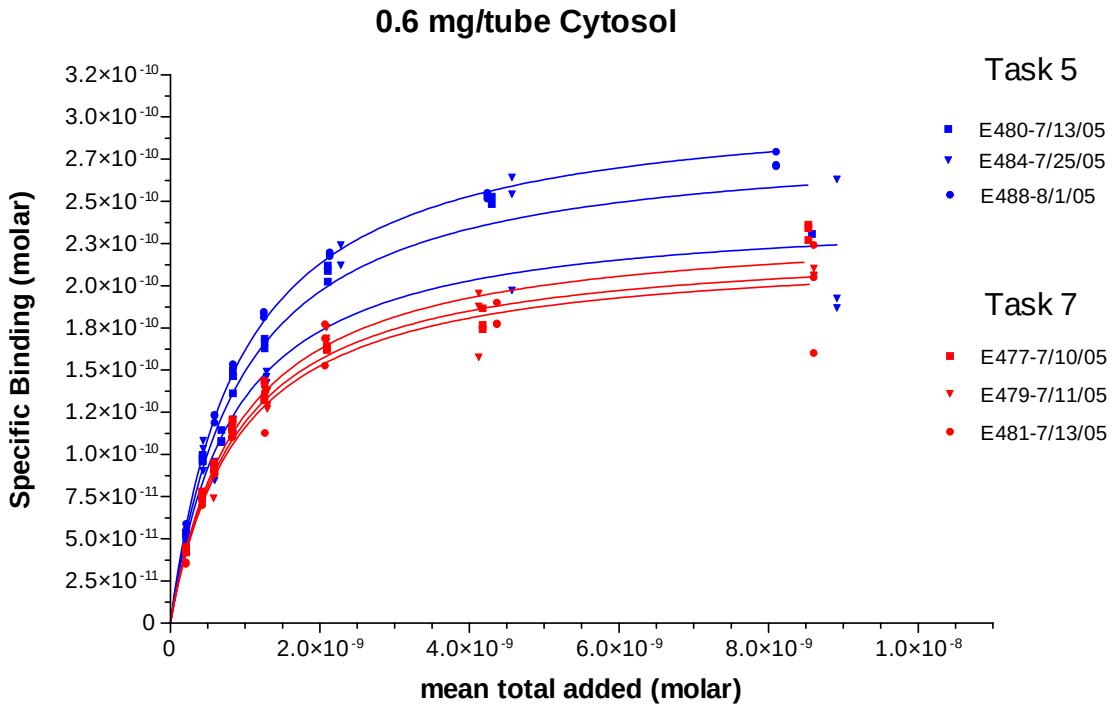

Figure 3. Intra-laboratory variability of one-site binding curves from Task 5 and 7 using individual laboratory prepared cytosol.

Competitive Binding Assay: Each participating laboratory conducted three independent runs of the competitive binding assay with a standard, a weak positive control, and ten test chemicals. Triplicate tubes of each concentration were performed during each run. The data used for the intra- -laboratory comparison are presented in the attached pdf file.

The goodness-of-fit to the one-site competition equation for the standard ranged from 0.30 to 1.00 with a median value of .99 for the 29 runs (Table 3). The low goodness-of-fit was associated with Lab C run C2-11/15/05. The percent bound was all less than 20% for this run (Figure 4). The goodness-of-fit for the weak positive control ranged from 0.02 to 0.99 with a median value of 0.96. Low goodness-of-fit was associated with several runs from Lab D (D5-11/29/05, D6-12/06/05, and D7-12/08/05 for which the percent bound was extremely variable for similar competitor concentrations (Figure 5). The range of IC_{50} values for the standard was 1.13E-09 to 6.54E-09 with a median value of 1.90E-09. The range of IC_{50} values for the weak positive control was 2.83E-05 to 9.64E-04 with a median value of 6.07E-05. The resulting RBAs ranged from 0.0004% to 0.0100% with a median value of 0.0039%. The intra-laboratory CV for RBA was 27% (Table 4). The variability in these measurements can be observed in the fitted one-site competitive curves for the standard and weak positive for all runs (Figures 6 and 7). Note, that curves for Lab B and Lab E would generally overlap.

Table 3. Intra-Laboratory variability of the statistics associated with the competitive assay for the standard and weak positive

Run	Standard Curve		Positive Control		
	IC50	R^2	IC50	R^2	RBA
Lab B					
B1-10/18/05	1.56E-09	0.99	6.33E-05	0.97	0.0025%
B2-10/20/05	1.60E-09	0.99	5.90E-05	0.97	0.0027%
B3-10/24/05	1.62E-09	0.99	6.07E-05	0.94	0.0027%
B4-10/26/05	1.31E-09	0.99	5.10E-05	0.96	0.0026%
B5-11/03/05	1.64E-09	0.99	5.68E-05	0.97	0.0029%
B6-11/07/05	1.54E-09	1.00	5.79E-05	0.99	0.0027%
B7-11/09/05	1.31E-09	0.99	4.79E-05	0.98	0.0027%
B8-11/14/05	1.13E-09	0.99	5.66E-05	0.96	0.0020%
Mean	1.46E-09	0.99	5.67E-05	0.97	0.0026%
CV	13%		9%		10%
Lab C					
C2-11/15/05	4.51E-09	0.30	8.16E-05	0.96	0.0055%
C3-11/28/05	5.16E-09	0.97	1.09E-04	0.96	0.0048%
C4-11/29/05	6.54E-09	0.85	1.31E-04	0.67	0.0050%
C5-11/16/05	2.10E-09	0.99	6.94E-05	0.98	0.0030%
C7-11/22/05	2.86E-09	0.99	7.12E-05	0.96	0.0040%
C8-12/19/05	1.83E-09	0.98	6.94E-05	0.95	0.0026%
Mean	3.83E-09	0.85	8.85E-05	0.92	0.0042%
CV	49%		29%		27%
Lab D					
D1-11/04/05	5.04E-09	0.95	1.52E-04	0.53	0.0033%
D2-11/08/05	4.58E-09	0.96	4.58E-05	0.88	0.0100%
D3-11/15/05	3.50E-09	0.95	7.31E-05	0.67	0.0048%
D4-11/22/05	4.61E-09	0.93	6.18E-05	0.75	0.0075%
D5-11/29/05	2.95E-09	0.96	1.07E-04	0.08	0.0028%
D6-12/06/05	3.47E-09	0.97	9.64E-04	0.02	0.0004%
D7-12/08/05	4.45E-09	0.89	8.09E-05	0.32	0.0055%
D8-12/08/05	3.08E-09	0.95	6.18E-05	0.84	0.0050%
D9-12/15/05	2.11E-09	0.97	5.38E-05	0.82	0.0039%
Mean	3.75E-09	0.95	1.78E-04	0.55	0.0048%
CV	26%		167%		58%
Lab E					
E517-11/08/05	1.52E-09	0.98	2.95E-05	0.96	0.0051%
E518-11/10/05	1.77E-09	0.99	2.84E-05	0.96	0.0062%
E519-11/17/05	1.46E-09	1.00	4.34E-05	0.99	0.0034%
E520-11/22/05	1.77E-09	1.00	4.00E-05	0.93	0.0044%
E522-12/08/05	1.90E-09	1.00	2.83E-05	0.94	0.0067%
E523-12/20/05	1.69E-09	1.00	3.36E-05	0.96	0.0050%
Mean	1.68E-09	0.99	3.39E-05	0.96	0.0051%
CV	10%		19%		24%

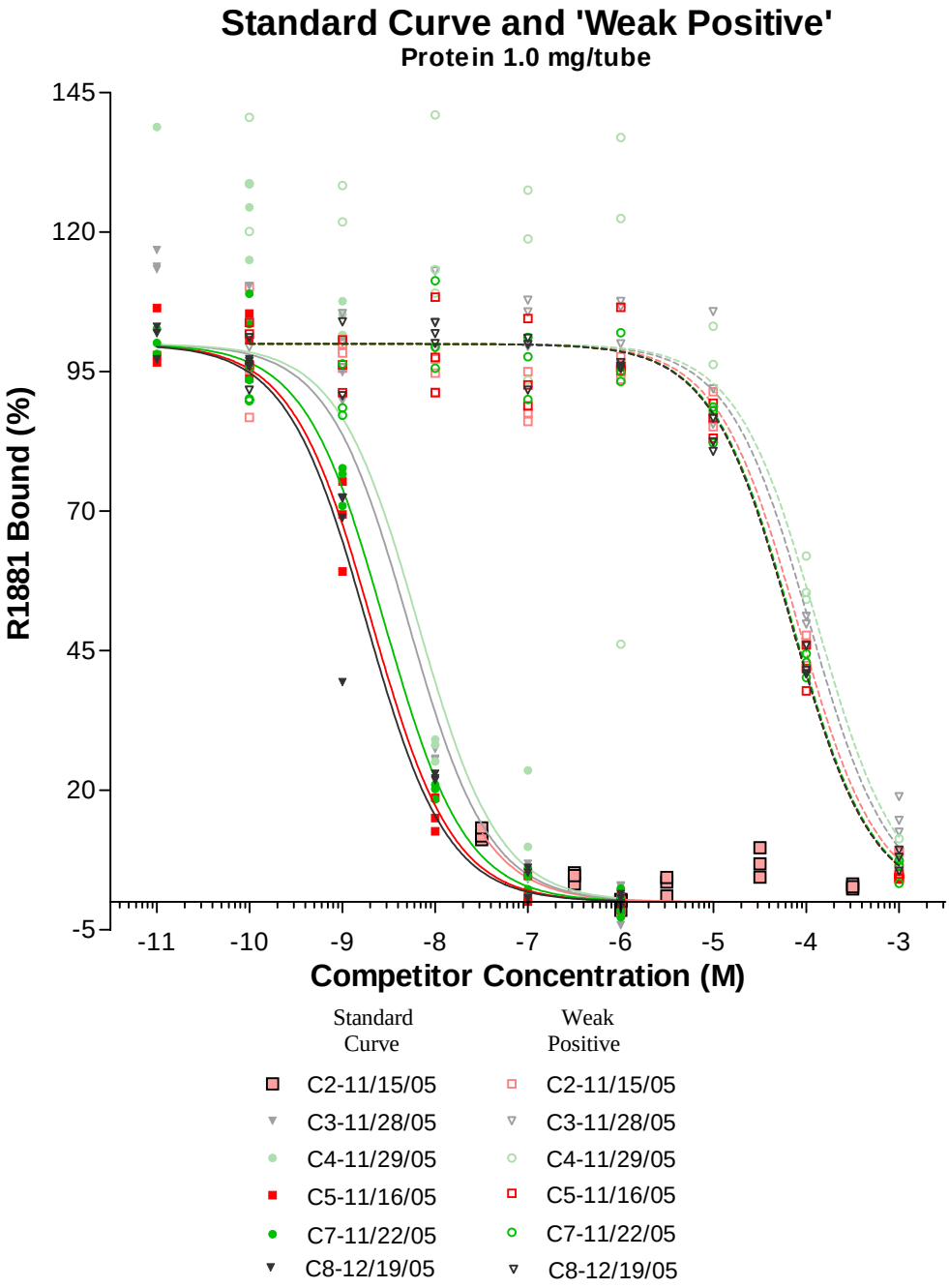

Figure 4. One-site competitive curves for the standard R1881 and weak positive for Lab C. Note run C2-11/15/05 had poor goodness-of-fit for the standard curve.

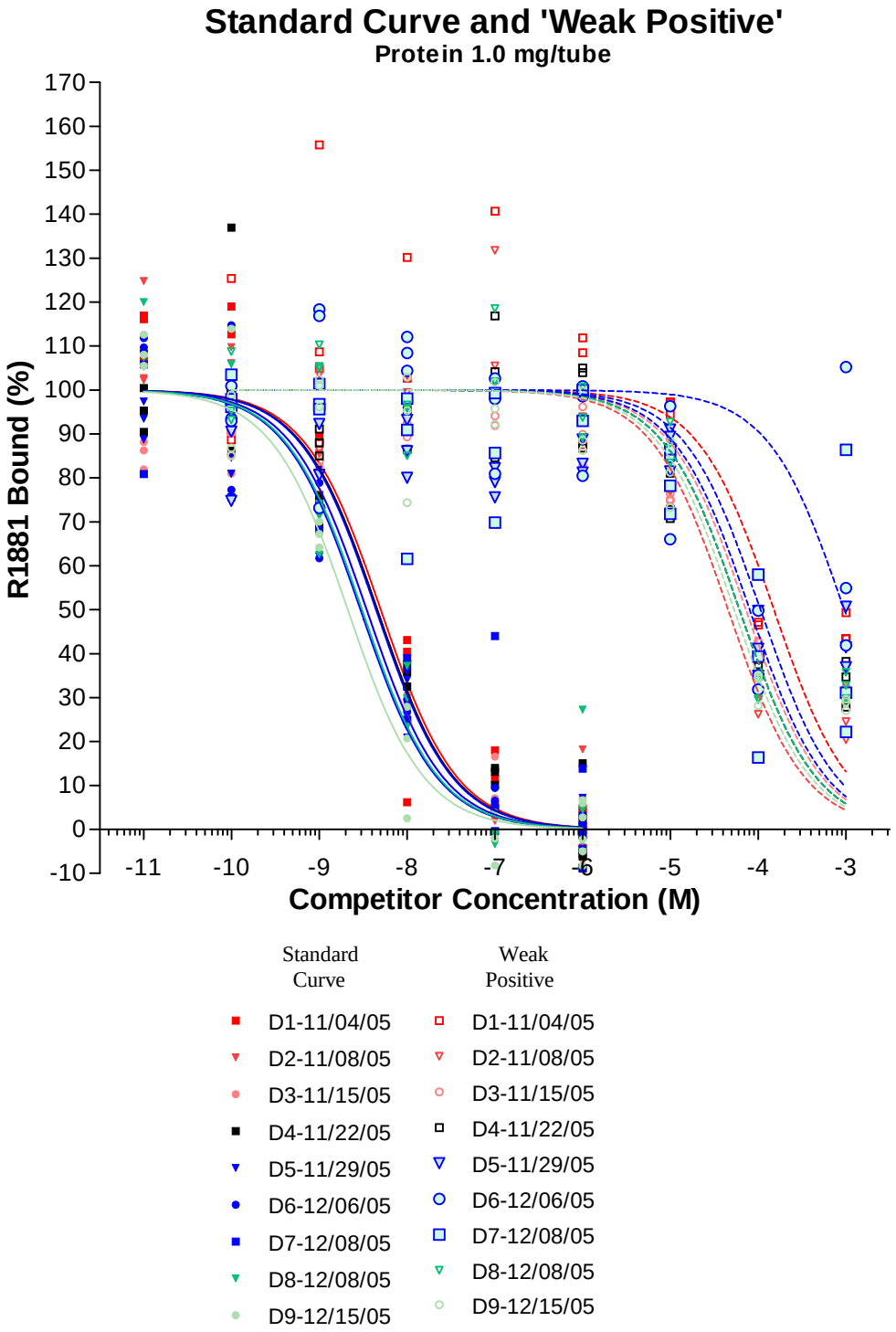

Figure 5. One-site competitive curves for the standard R1881 and weak positive for Lab D. Note runs D5, D6, and D7 had poor goodness-of-fit for the weak positive curves.

Table 4. Inter-Laboratory variability of the statistics associated with the competitive binding assay

Statistic	IC50 Standard	IC50 Weak Positive	RBA
Mean	2.68E-09	8.92E-05	0.0042%
CV	48%	71%	27%

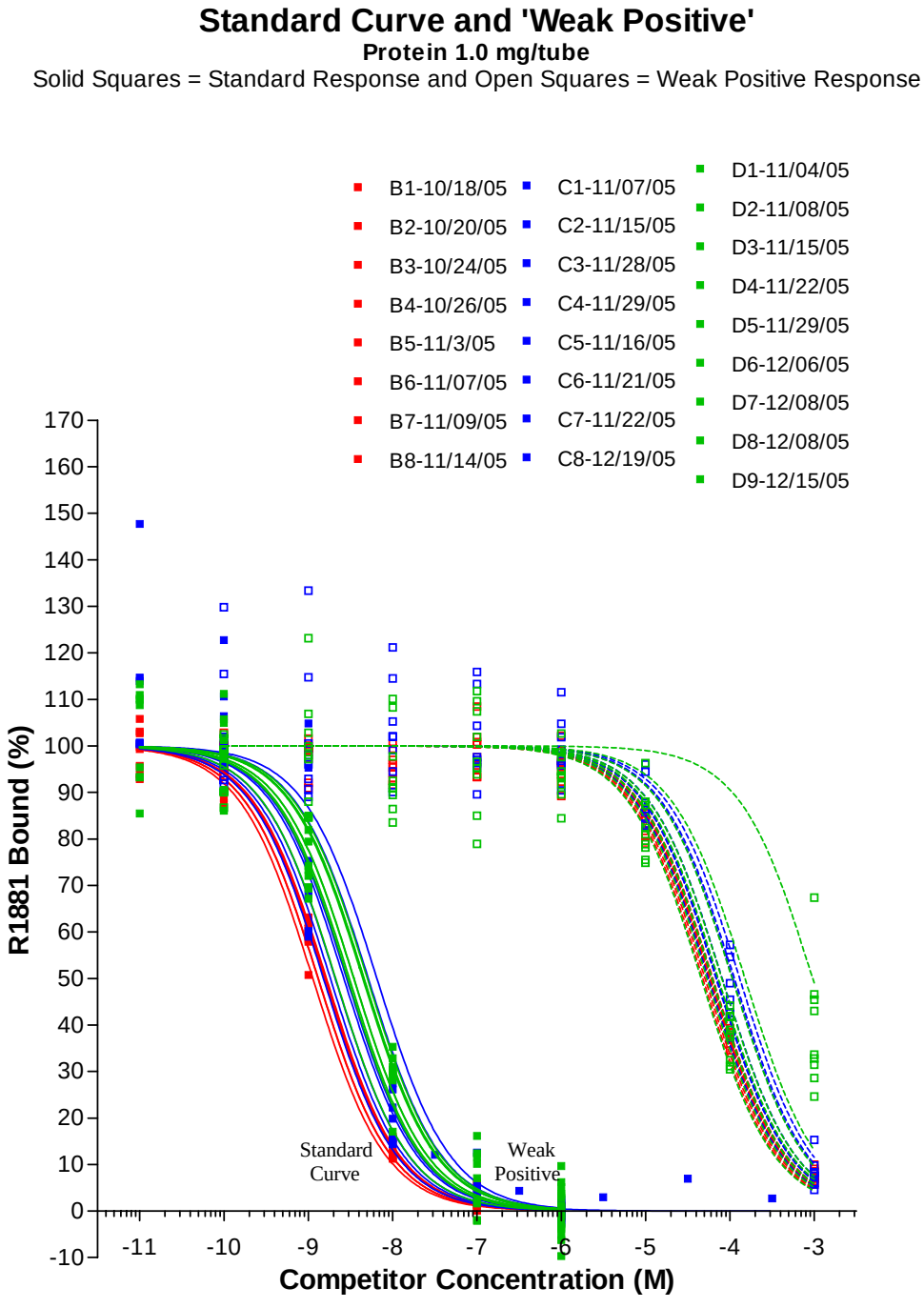

Figure 6. One-site competitive curves for the standard R1881 and weak positive for Labs B, C, and D.

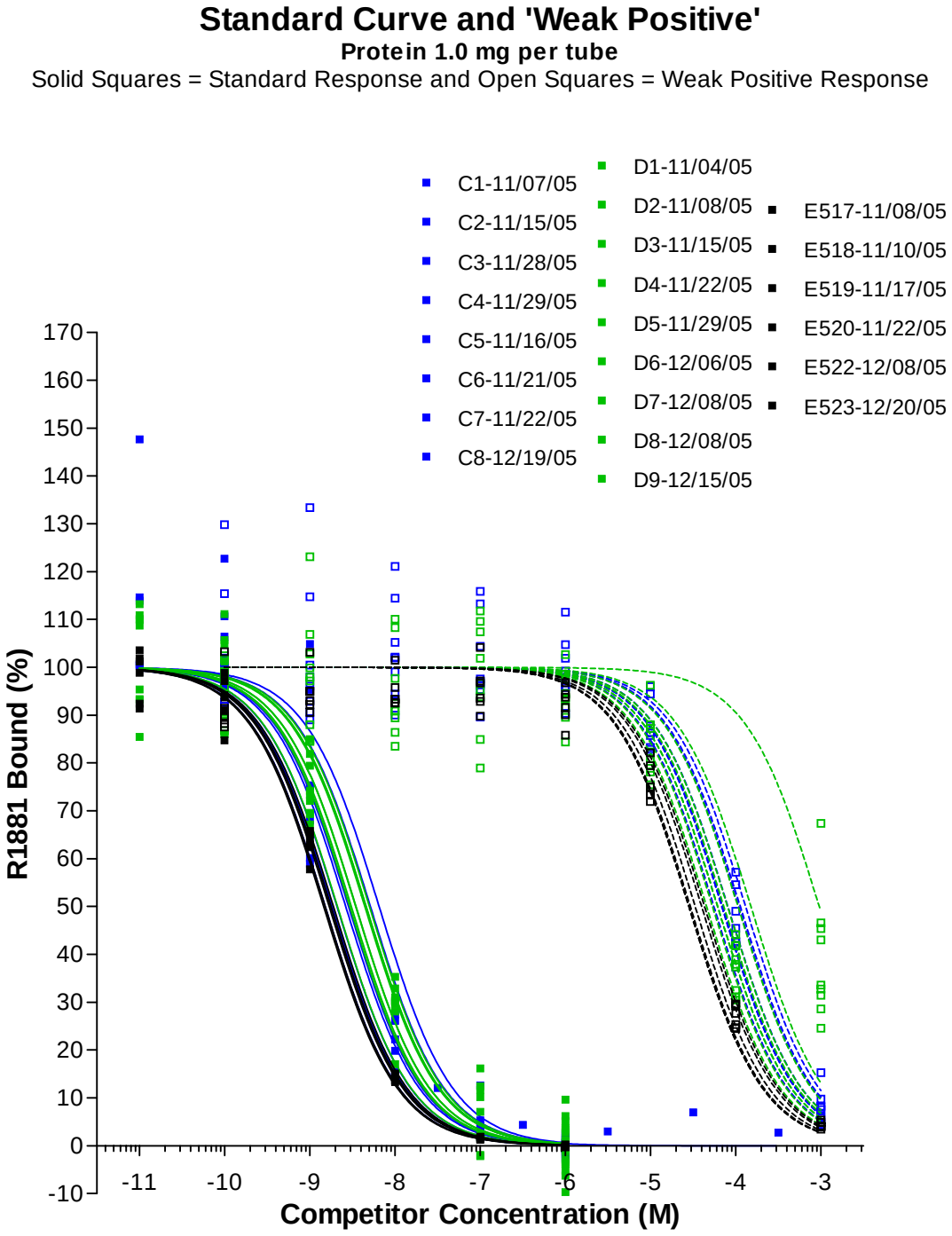

Figure 7. One-site competitive curves for the standard R1881 and weak positive for Labs C, D, and E.

The goodness-of-fit to the one-site competitive equation for the ten test chemicals reflected the characteristics of the chemical being tested (Tables 4-8). Thus, the lack of convergence did not cause any data to be removed from the analysis. Most (80%) of the R^2 values were greater than 80%. One run of CR42404, linuron, from Lab C and Lab D either did not converge or produced a poor fit to the curve (R^2 values less than or equal to 0.4), but the other two runs in both cases had R^2 values greater than 0.5. One run of CR42406, cyproterone acetate, from Lab D had a poor fit to the curve (R2 = 0.32), but again this was an anomoly. Chemicals CR42408, bis(2-ethylhexyl) phthalate (DEPH), and CR42409, atrazine, either did not converge or fit the curve poorly for nearly all laboratories and runs.

The mean IC_{50}, R^2, and RBA and intra-laboratory CVs for the test chemicals are presented in each of the Tables 4-8. The intra-laboratory CVs for the test chemical RBAs ranged from 4% to 136% with a median of 19%. The large CV of 136% was directly related to a highly variable run of CR42402, 17β−estradiol. from Lab D. The CVs for RBA were generally less than 60%, and there was no apparent pattern (Figure 8). The competitive curves for each chemical and its associated standard and weak positive are presented in Figures 9-18.

Table 4. Intra-Laboratory Competitive Assay Results for Associated Runs of the Standard, Weak Positive, and Test Chemicals CR42400 and CR42401

Run	Standard Curve		Positive Control			CR42400 5a-Androstan-17B-ol-3-one (4,5a-Dihydrotestosterone)			CR42401 Testosterone		
	IC50	R^2	IC50	R^2	RBA	IC50	R^2	RBA	IC50	R^2	RBA
B1-10/18/05	1.56E-09	0.99	6.33E-05	0.97	0.0025%	9.92E-10	0.99	158%	7.70E-09	0.97	20.3%
B4-10/26/05	1.31E-09	0.99	5.10E-05	0.96	0.0026%	1.14E-09	1.00	114%	7.34E-09	1.00	17.8%
B6-11/07/05	1.54E-09	1.00	5.79E-05	0.99	0.0027%	1.06E-09	1.00	145%	8.27E-09	1.00	18.6%
					Mean	1.07E-09	0.99	139%	7.77E-09	0.99	18.9%
					CV	7.2%		16%	6.0%		6.8%
C2-11/15/05	4.51E-09	0.30	8.16E-05	0.96	0.0055%	2.36E-09	0.94	191%	1.97E-08	0.98	22.9%
C3-11/28/05	5.16E-09	0.97	1.09E-04	0.96	0.0048%	2.86E-09	0.87	181%	1.40E-08	0.98	36.8%
C4-11/29/05	6.54E-09	0.85	1.31E-04	0.67	0.0050%	4.94E-09	0.78	132%	3.07E-08	0.94	21.3%
					Mean	3.39E-09	0.86	168%	2.15E-08	0.97	27.0%
					CV	40%		19%	39%		31%
D1-11/04/05	5.04E-09	0.95	1.52E-04	0.53	0.0033%	7.07E-09	0.75	71.2%	2.30E-08	0.84	21.9%
D2-11/08/05	4.58E-09	0.96	4.58E-05	0.88	0.0100%	3.24E-09	0.93	141%	2.09E-08	0.96	21.9%
D3-11/15/05	3.50E-09	0.95	7.31E-05	0.67	0.0048%	4.89E-09	0.98	71.5%	2.05E-08	0.99	17.0%
					Mean	5.07E-09	0.89	94.7%	2.15E-08	0.93	20.3%
					CV	38%		43%	6.2%		14%
E517-11/08/05	1.52E-09	0.98	2.95E-05	0.96	0.0051%	1.61E-09	0.99	94.2%	7.22E-09	1.00	21.0%
E518-11/10/05	1.77E-09	0.99	2.84E-05	0.96	0.0062%	2.05E-09	0.98	86.3%	9.57E-09	0.99	18.5%
E519-11/17/05	1.46E-09	1.00	4.34E-05	0.99	0.0034%	2.27E-09	1.00	64.2%	9.22E-09	1.00	15.8%
					Mean	1.98E-09	0.99	81.6%	8.67E-09	1.00	18.4%
					CV	17%		19%	15%		14%

Table 5. Intra-Laboratory Competitive Assay Results for Associated Runs of the Standard, Weak Positive, and Test Chemicals CR42402 and CR42403

Run	Standard Curve		Positive Control			CR42402 17B-Estradiol			CR42403 6a-Methyl-17a-hydroxyprogesterone acetate (MPA)		
	IC50	R^2	IC50	R^2	RBA	IC50	R^2	RBA	IC50	R^2	RBA
B1-10/18/05	1.56E-09	0.99	6.33E-05	0.97	0.0025%	2.88E-07	0.99	0.543%			
B2-10/20/05	1.60E-09	0.99	5.90E-05	0.97	0.0027%				2.27E-08	0.98	7.03%
B4-10/26/05	1.31E-09	0.99	5.10E-05	0.96	0.0026%	2.38E-07	0.99	0.549%	1.67E-08	0.99	7.81%
B7-11/09/05	1.31E-09	0.99	4.79E-05	0.98	0.0027%	1.89E-07	1.00	0.695%	1.44E-08	0.99	9.14%
					Mean	2.38E-07	0.99	0.596%	1.79E-08	0.98	7.99%
					CV	21%		14%	24%		13%
C2-11/15/05	4.51E-09	0.30	8.16E-05	0.96	0.0055%	4.54E-07	0.99	0.993%	4.37E-08	0.98	10.3%
C3-11/28/05	5.16E-09	0.97	1.09E-04	0.96	0.0048%	4.13E-07	0.99	1.25%	3.69E-08	0.99	14.0%
C4-11/29/05	6.54E-09	0.85	1.31E-04	0.67	0.0050%	5.26E-07	0.98	1.24%	5.09E-08	0.76	12.8%
					Mean	4.64E-07	0.99	1.16%	4.38E-08	0.91	12.4%
					CV	12%		13%	16%		15%
D1-11/04/05	5.04E-09	0.95	1.52E-04	0.53	0.0033%	1.53E-07	0.66	3.29%			
D2-11/08/05	4.58E-09	0.96	4.58E-05	0.88	0.0100%	1.01E-06	0.83	0.453%			
D3-11/15/05	3.50E-09	0.95	7.31E-05	0.67	0.0048%	3.14E-06	0.70	0.112%			
D4-11/22/05	4.61E-09	0.93	6.18E-05	0.75	0.0075%				1.30E-07	0.96	3.54%
D5-11/29/05	2.95E-09	0.96	1.07E-04	0.08	0.0028%				1.35E-07	0.97	2.19%
D6-12/06/05	3.47E-09	0.97	9.64E-04	0.02	0.0004%				3.05E-07	0.92	1.14%
					Mean	1.43E-06	0.73	1.29%	1.90E-07	0.95	2.29%
					CV	107%		136%	52%		53%
E517-11/08/05	1.52E-09	0.98	2.95E-05	0.96	0.0051%	3.05E-07	1.00	0.498%			
E518-11/10/05	1.77E-09	0.99	2.84E-05	0.96	0.0062%	2.84E-07	0.99	0.624%			
E519-11/17/05	1.46E-09	1.00	4.34E-05	0.99	0.0034%	4.14E-07	0.99	0.351%			
E520-11/22/05	1.77E-09	1.00	4.00E-05	0.93	0.0044%				1.74E-08	1.00	10.1%
E522-12/08/05	1.90E-09	1.00	2.83E-05	0.94	0.0067%				1.49E-08	0.99	12.7%
E523-12/20/05	1.69E-09	1.00	3.36E-05	0.96	0.0050%				1.68E-08	1.00	10.1%
					Mean	3.34E-07	0.99	0.491%	1.64E-08	0.99	11.0%
					CV	21%		28%	7.8%		13%

Table 6. Intra-Laboratory Competitive Assay Results for Associated Runs of the Standard, Weak Positive, and Test Chemicals CR42404 and CR42405

Run	Standard Curve		Positive Control			CR42404 Linuron			CR42405 1,1-Dichloro-2,2-bis(4-chlorophenyl) ethane (pp'-DDE)		
	IC50	R^2	IC50	R^2	RBA	IC50	R^2	RBA	IC50	R^2	RBA
B2-10/20/05	1.60E-09	0.99	5.90E-05	0.97	0.0027%	9.18E-05	0.96	0.0017%	1.70E-05	0.94	0.0094%
B5-11/03/05	1.64E-09	0.99	5.68E-05	0.97	0.0029%	1.11E-04	0.97	0.0015%	1.65E-05	0.87	0.0099%
B7-11/09/05	1.31E-09	0.99	4.79E-05	0.98	0.0027%	7.73E-05	0.98	0.0017%	1.14E-05	0.90	0.0115%
					Mean	9.32E-05	0.97	0.0016%	1.50E-05	0.91	0.0103%
					CV	18%		8.3%	21%		11%
C2-11/15/05	4.51E-09	0.30	8.16E-05	0.96	0.0055%	1.30E-04	0.97	0.0035%			
C3-11/28/05	5.16E-09	0.97	1.09E-04	0.96	0.0048%	1.11E-04	0.98	0.0047%			
C4-11/29/05	6.54E-09	0.85	1.31E-04	0.67	0.0050%	4.96E-05	0.15	0.0132%			
C5-11/16/05	2.10E-09	0.99	6.94E-05	0.98	0.0030%				1.11E-05	0.96	0.0188%
C7-11/22/05	2.86E-09	0.99	7.12E-05	0.96	0.0040%				1.84E-05	0.70	0.0156%
C8-12/19/05	1.83E-09	0.98	6.94E-05	0.95	0.0026%				1.43E-05	0.93	0.0128%
					Mean	9.66E-05	0.70	0.0071%	1.46E-05	0.86	0.0157%
					CV	43%		75%	25%		19%
D4-11/22/05	4.61E-09	0.93	6.18E-05	0.75	0.0075%	9.80E-05	0.78	0.0047%	5.38E-06	0.95	0.0856%
D5-11/29/05	2.95E-09	0.96	1.07E-04	0.08	0.0028%	**Did not Converge**			9.23E-06	0.95	0.0320%
D6-12/06/05	3.47E-09	0.97	9.64E-04	0.02	0.0004%	2.04E-04	0.58	0.0017%	1.81E-05	0.88	0.0192%
					Mean	1.51E-04	0.68	0.0032%	1.09E-05	0.93	0.0456%
					CV	50%		66%	60%		77%
E517-11/08/05	1.52E-09	0.98	2.95E-05	0.96	0.0051%	7.41E-05	0.97	0.0020%	1.56E-05	0.99	0.0098%
E518-11/10/05	1.77E-09	0.99	2.84E-05	0.96	0.0062%	9.46E-05	0.94	0.0019%	1.56E-05	0.97	0.0114%
E519-11/17/05	1.46E-09	1.00	4.34E-05	0.99	0.0034%	1.02E-04	0.87	0.0014%	1.84E-05	0.99	0.0079%
					Mean	9.02E-05	0.93	0.0018%	1.65E-05	0.98	0.0097%
					CV	16%		18%	9.9%		18%

Table 7. Intra-Laboratory Competitive Assay Results for Associated Runs of the Standard, Weak Positive, and Test Chemicals CR42406 and CR42407

Run	Standard Curve		Positive Control			CR42406 Cyproterone Acetate			CR42407 Spironolactone		
	IC50	R^2	IC50	R^2	RBA	IC50	R^2	RBA	IC50	R^2	RBA
B3-10/24/05	1.62E-09	0.99	6.07E-05	0.94	0.0027%	1.44E-07	1.00	1.13%	2.79E-07	0.99	0.580%
B5-11/03/05	1.64E-09	0.99	5.68E-05	0.97	0.0029%	1.64E-07	0.99	1.00%	3.06E-07	0.99	0.536%
B8-11/14/05	1.13E-09	0.99	5.66E-05	0.96	0.0020%	1.33E-07	0.99	0.853%	3.16E-07	0.98	0.359%
					Mean	1.47E-07	0.99	0.994%	3.00E-07	0.99	0.492%
					CV	11%		14%	6.4%		24%
C5-11/16/05	2.10E-09	0.99	6.94E-05	0.98	0.0030%	2.12E-07	0.99	0.988%	3.59E-07	1.00	0.584%
C7-11/22/05	2.86E-09	0.99	7.12E-05	0.96	0.0040%	1.67E-07	0.99	1.72%	2.94E-07	0.99	0.973%
C8-12/19/05	1.83E-09	0.98	6.94E-05	0.95	0.0026%	3.11E-07	0.98	0.591%	4.69E-07	0.99	0.391%
					Mean	2.30E-07	0.99	1.10%	3.74E-07	0.99	0.650%
					CV	32%		52%	24%		46%
D7-12/08/05	4.45E-09	0.89	8.09E-05	0.32	0.0055%	5.39E-07	0.95	0.824%	8.39E-07	0.79	0.530%
D8-12/08/05	3.08E-09	0.95	6.18E-05	0.84	0.0050%	7.30E-07	0.95	0.422%	8.12E-07	0.87	0.379%
D9-12/15/05	2.11E-09	0.97	5.38E-05	0.82	0.0039%	4.90E-07	0.32	0.432%	8.42E-07	0.87	0.251%
					Mean	5.86E-07	0.74	0.559%	8.31E-07	0.84	0.387%
					CV	22%		41%	2.0%		36%
E520-11/22/05	1.77E-09	1.00	4.00E-05	0.93	0.0044%	1.33E-07	1.00	1.33%	2.34E-07	0.99	0.755%
E522-12/08/05	1.90E-09	1.00	2.83E-05	0.94	0.0067%	8.99E-08	0.99	2.11%	2.30E-07	0.98	0.825%
E523-12/20/05	1.69E-09	1.00	3.36E-05	0.96	0.0050%	1.20E-07	0.99	1.41%	2.51E-07	1.00	0.673%
					Mean	1.14E-07	0.99	1.61%	2.38E-07	0.99	0.751%
					CV	19%		27%	4.8%		10%

Table 8. Intra-Laboratory Competitive Assay Results for Associated Runs of the Standard, Weak Positive, and Test Chemicals CR42408 and CR42409

Run	Standard Curve		Positive Control			CR42408 Bis(2-ethylhexyl) phthalate (DEPH)			CR42409 Atrazine		
	IC50	R^2	IC50	R^2	RBA	IC50	R^2	RBA	IC50	R^2	RBA
B3-10/24/05	1.62E-09	0.99	6.07E-05	0.94	0.0027%	**Did not Converge**			8.83E-03	0.33	0.0000%
B6-11/07/05	1.54E-09	1.00	5.79E-05	0.99	0.0027%	**Did not Converge**			8.84E-03	0.20	0.0000%
B8-11/14/05	1.13E-09	0.99	5.66E-05	0.96	0.0020%	**Did not Converge**			**Did not Converge**		
					Mean	**Did not Converge**			8.84E-03	0.27	0.0000%
					CV	NA		NA	0.06%		3.5%
C5-11/16/05	2.10E-09	0.99	6.94E-05	0.98	0.0030%	2.63E-02	0.03	0.0000%	5.58E-03	0.41	0.0000%
C7-11/22/05	2.86E-09	0.99	7.12E-05	0.96	0.0040%	**Did not Converge**			**Did not Converge**		
C8-12/19/05	1.83E-09	0.98	6.94E-05	0.95	0.0026%	**Did not Converge**			**Did not Converge**		
					Mean	2.63E-02	0.03	0.0000%	5.58E-03	0.41	0.0000%
					CV	NA		NA	NA		NA
D7-12/08/05	4.45E-09	0.89	8.09E-05	0.32	0.0055%	1.57E-03	0.27	0.0003%	1.24E-03	0.51	0.0004%
D8-12/08/05	3.08E-09	0.95	6.18E-05	0.84	0.0050%	5.28E-03	0.04	0.0001%	**Did not Converge**		
D9-12/15/05	2.11E-09	0.97	5.38E-05	0.82	0.0039%	**Did not Converge**			**Did not Converge**		
					Mean	3.43E-03	0.16	0.0002%	1.24E-03	0.51	0.0004%
					CV	77%		93%	NA		NA
E520-11/22/05	1.77E-09	1.00	4.00E-05	0.93	0.0044%	7.85E-03	0.52	0.0000%	4.65E-03	0.44	0.0000%
E522-12/08/05	1.90E-09	1.00	2.83E-05	0.94	0.0067%	**Did not Converge**			7.90E-03	0.33	0.0000%
E523-12/20/05	1.69E-09	1.00	3.36E-05	0.96	0.0050%	1.46E-02	0.34	0.0000%	6.17E-03	0.86	0.0000%
					Mean	1.12E-02	0.43	0.0000%	6.24E-03	0.54	0.0000%
					CV	42%		45%	26%		24%

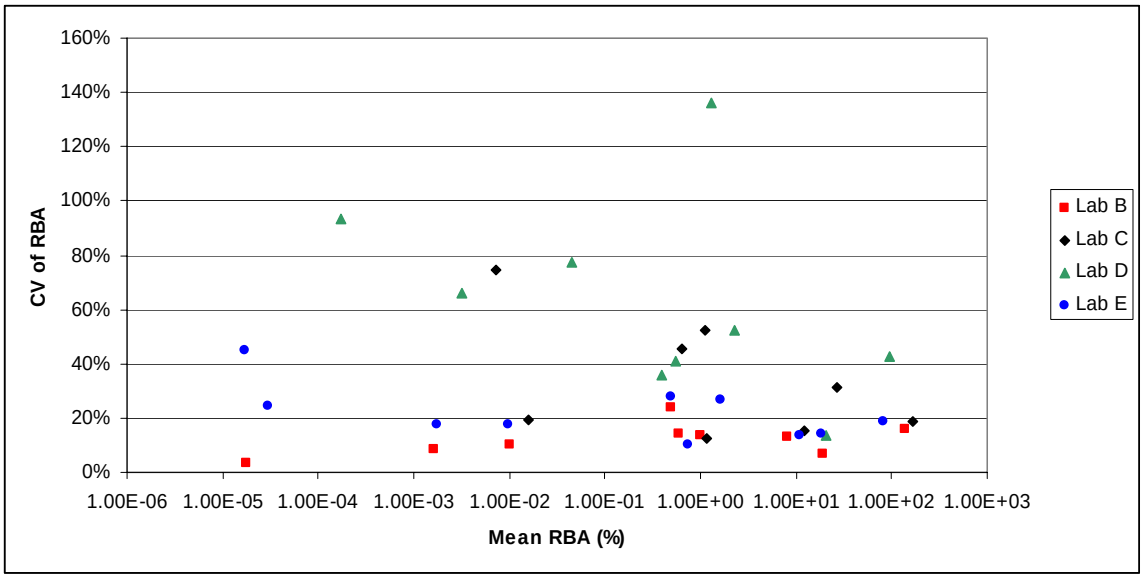

Figure 8. CV and associated RBA of 10 test chemicals.

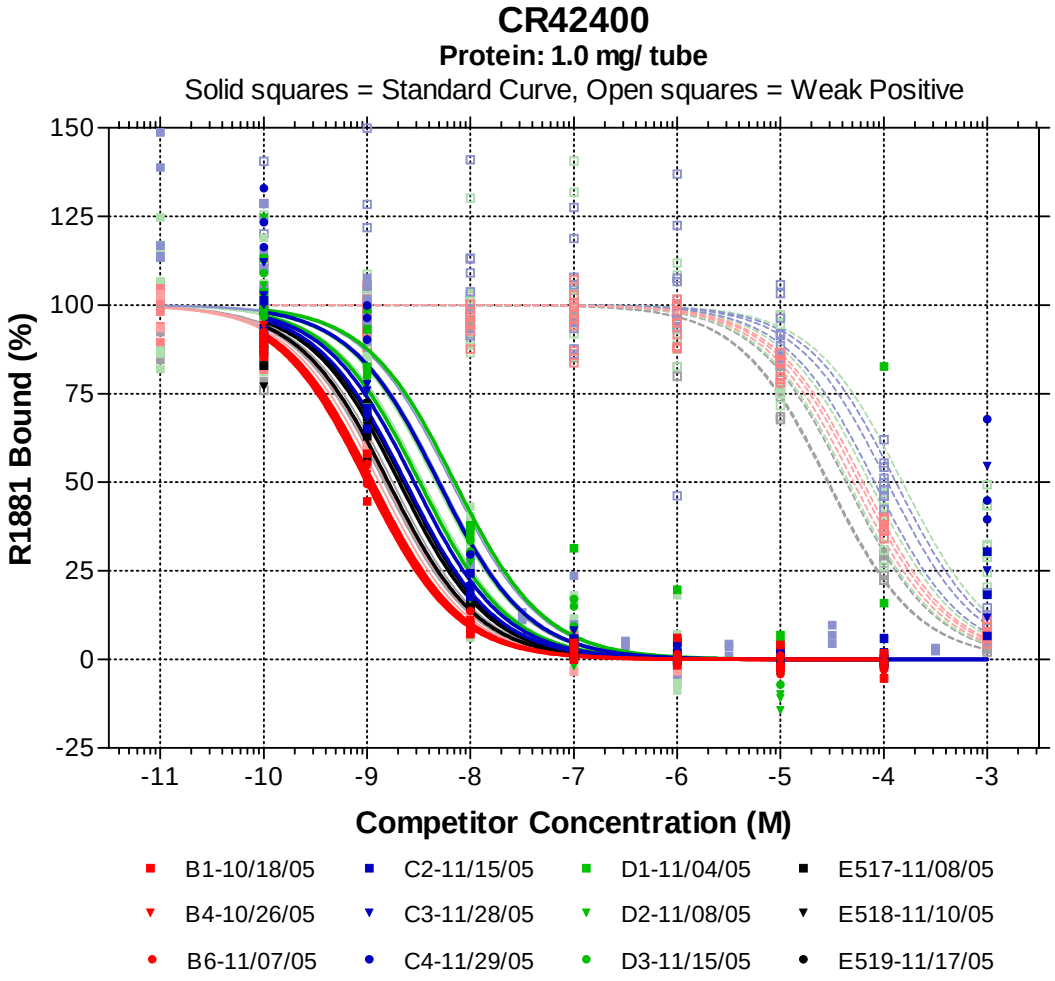

Figure 9. Inter-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42400 = 5a-Androstan-17B-ol-3-one (4,5a-Dihydrotestosterone)

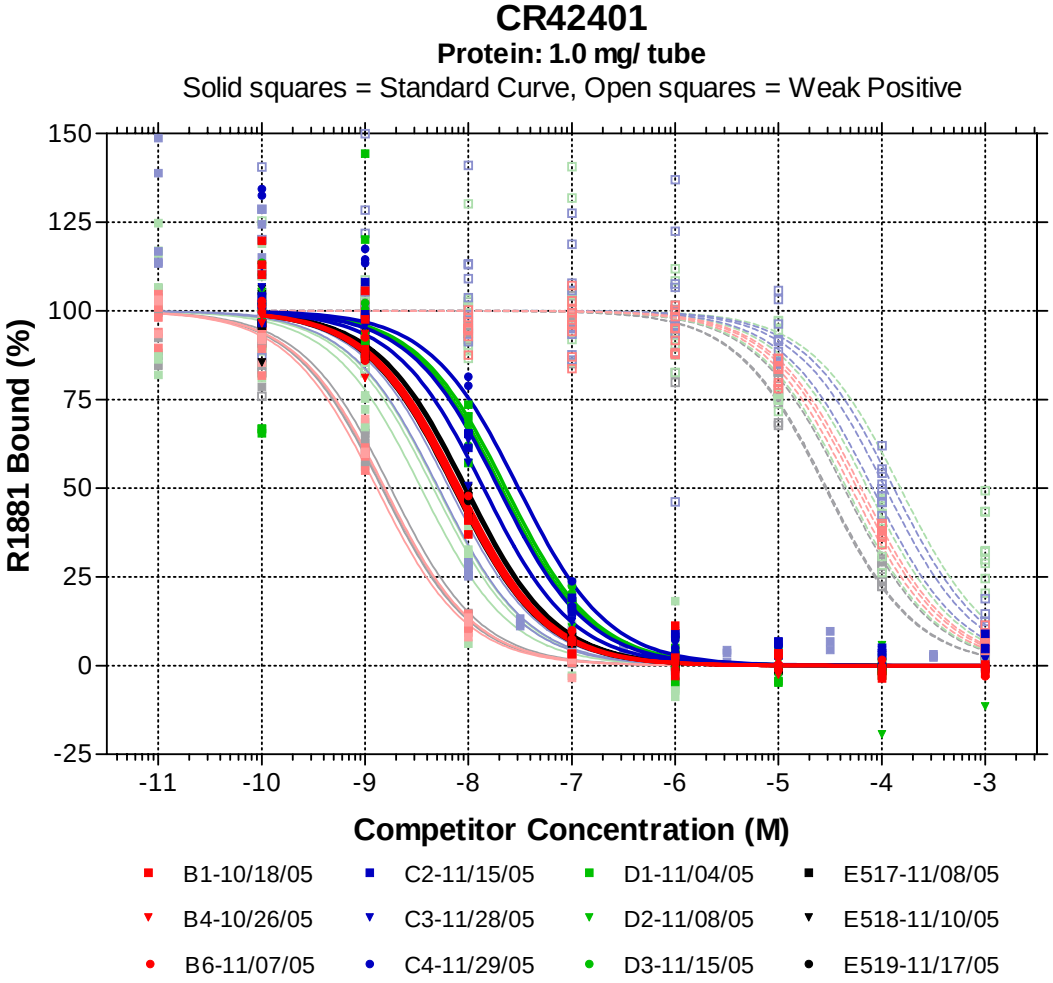

Figure 10. Inter-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42401 = Testosterone

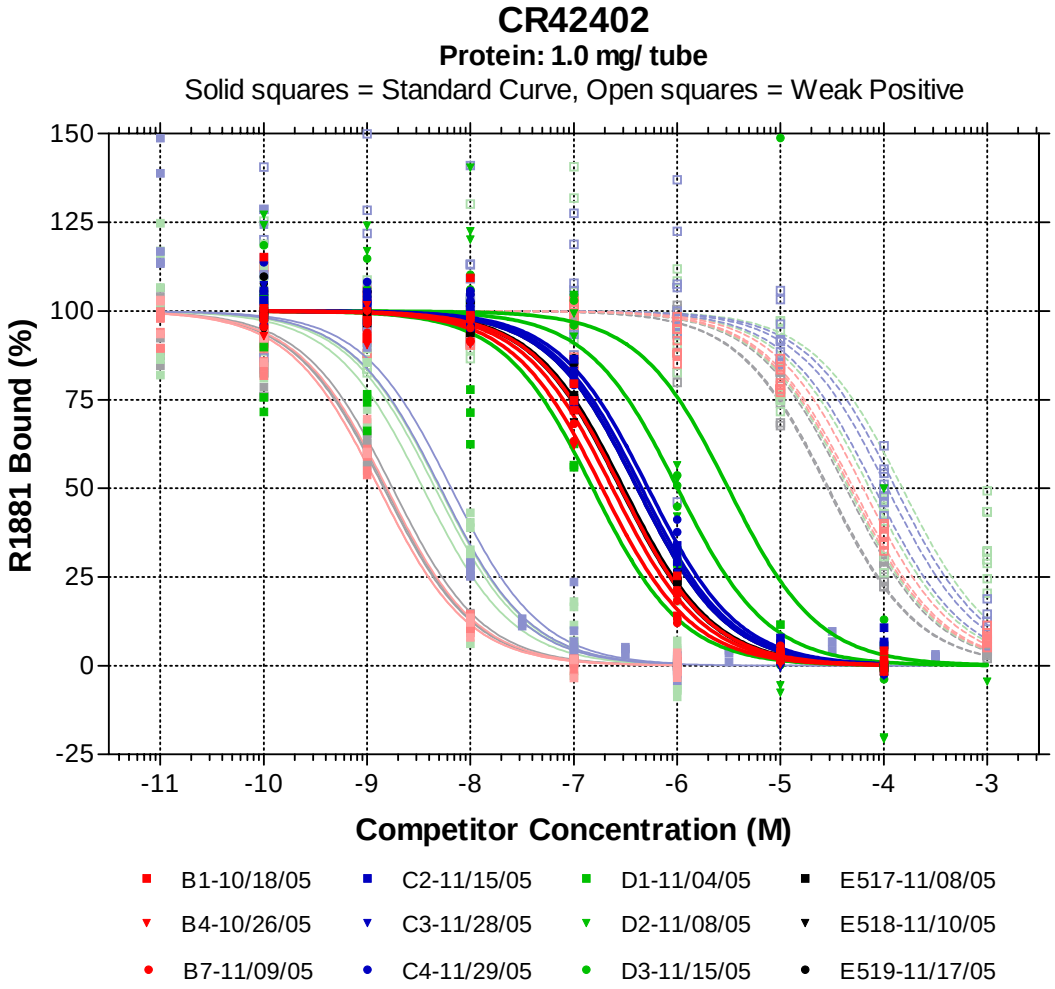

Figure 11. Inter-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42402 = 17β-Estradiol

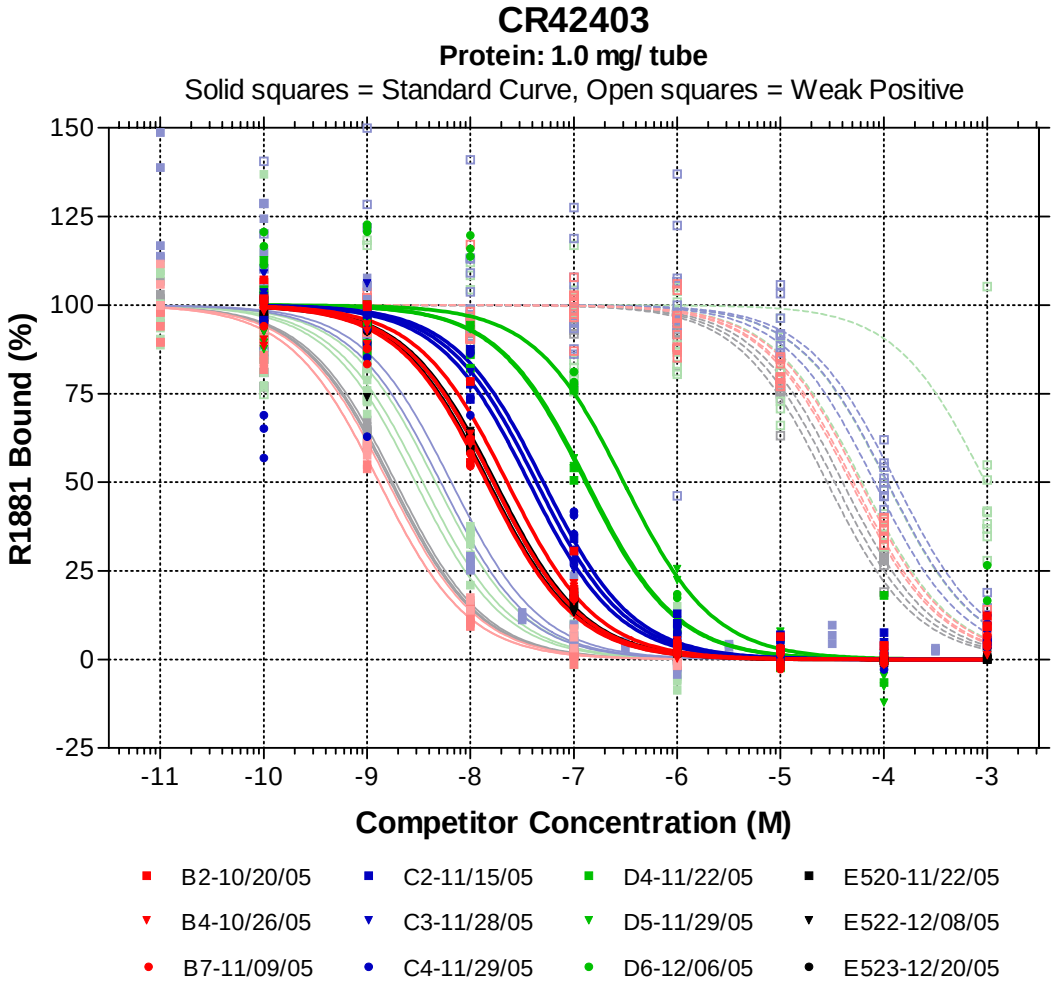

Figure 12. Inter-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42403 = 6a-Methyl-17a-hydroxyprogesterone acetate (MPA)

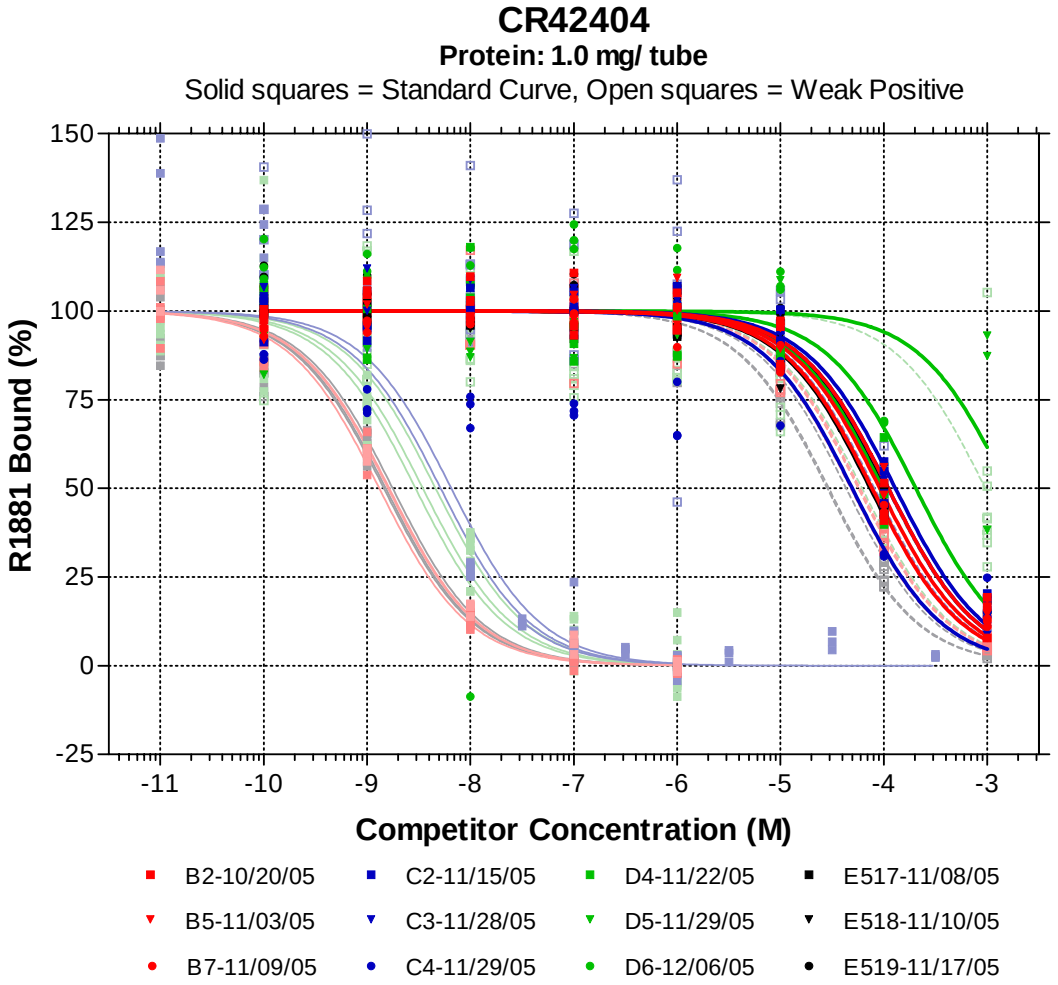

Figure 13. Inter-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42404 = Linuron

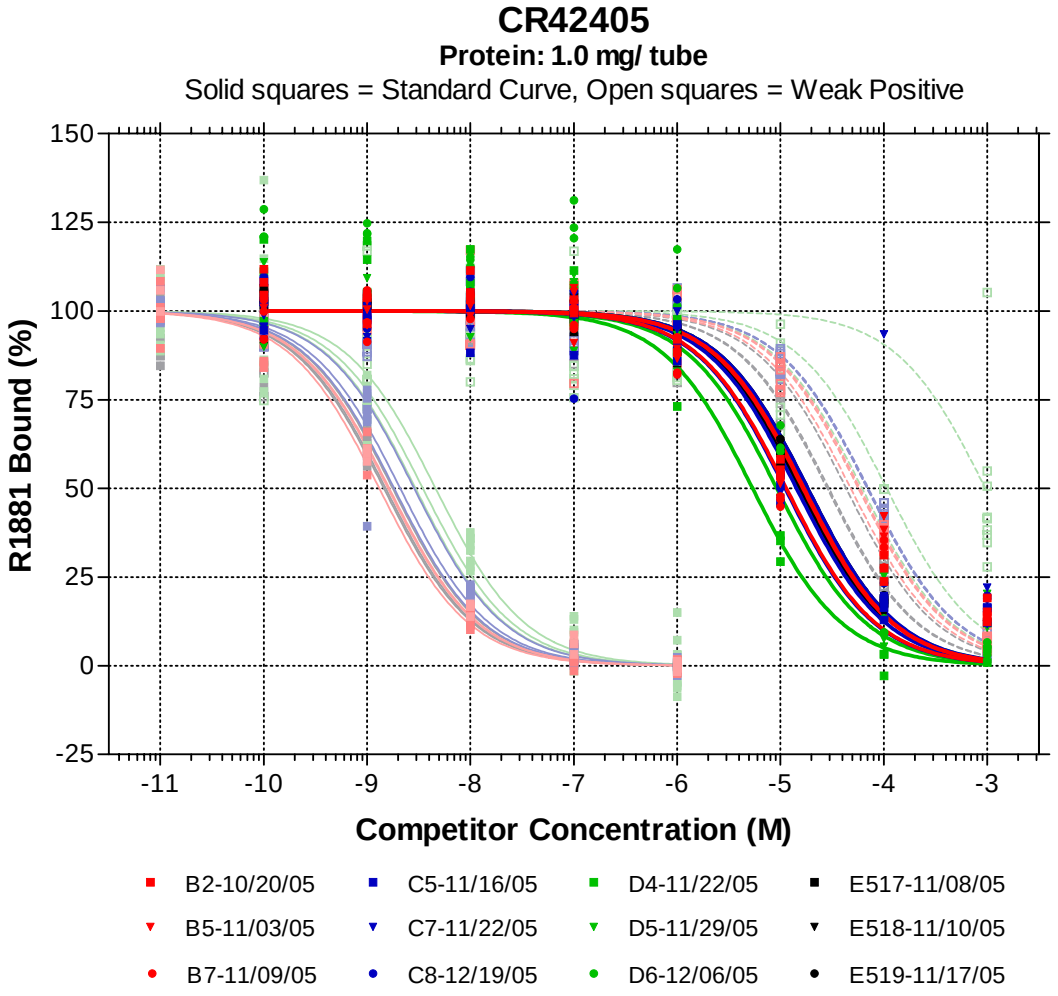

Figure 14. Inter-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42405 = 1,1-Dichloro-2,2-bis(4-chlorophenyl)ethane (pp'-DDE)

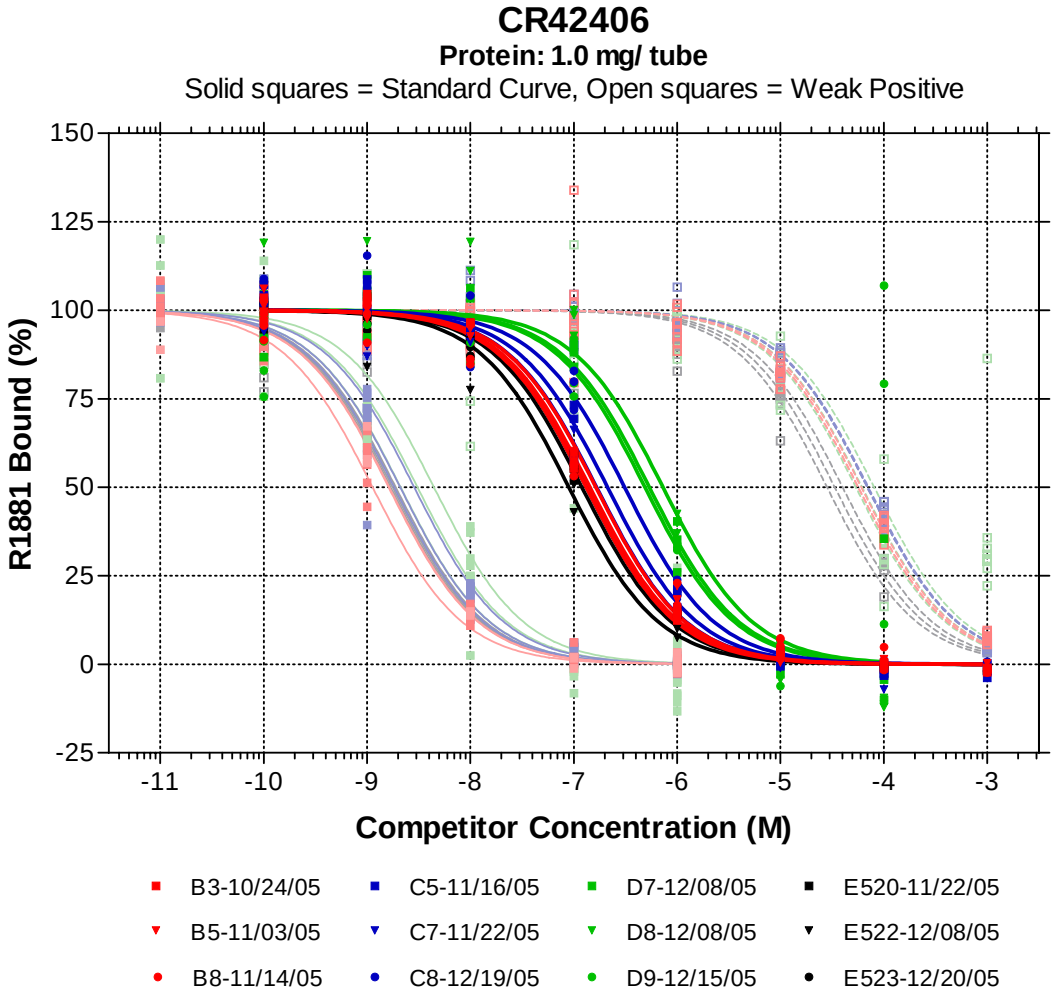

Figure 15. Inter-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42406 = Cyproterone Acetate

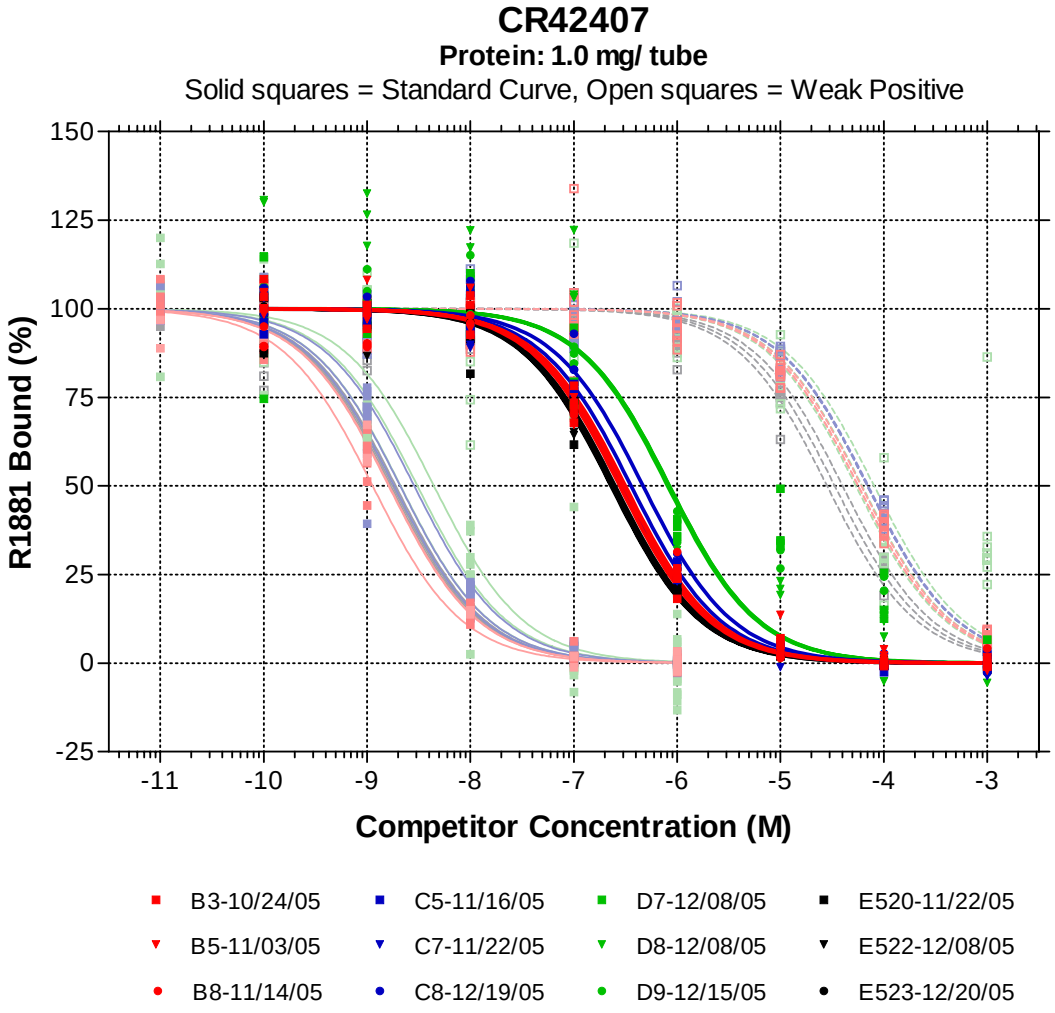

Figure 16. Inter-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42407 = Spironolactone

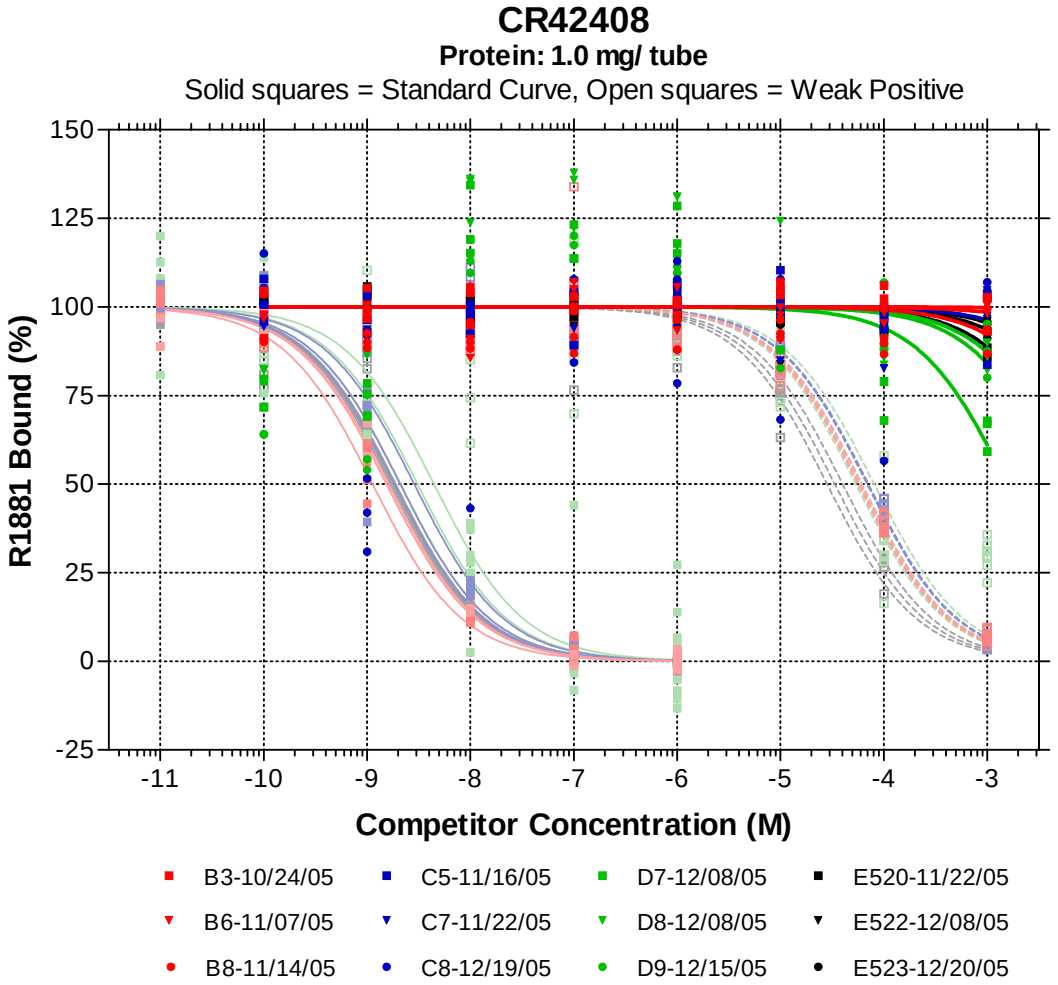

Figure 17. Inter-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42408 = Bis(2-ethylhexyl) phthalate (DEPH)

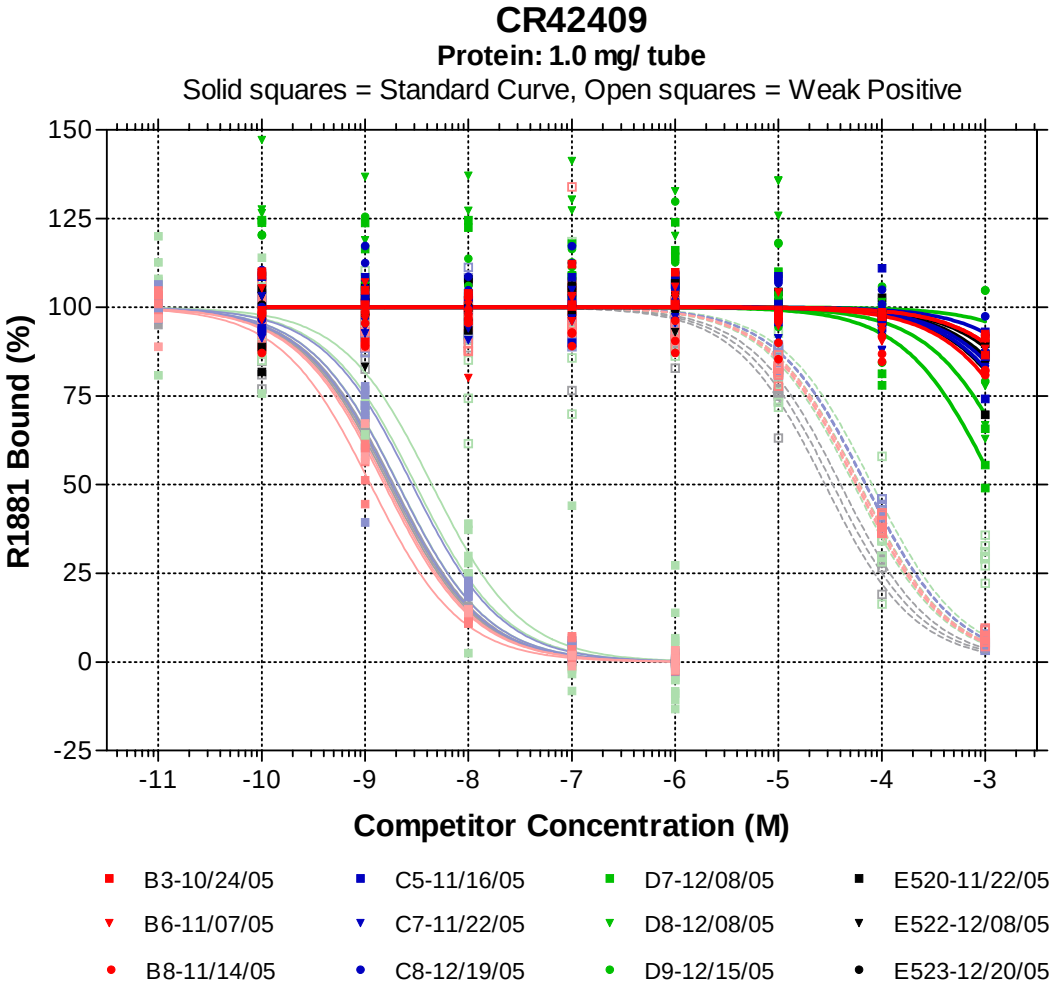

Figure 18. Inter-Laboratory Variability of the Competitive Binding Curves for the Standard and Weak Positive (muted colors), and Test Chemical CR42409 = Atrazine

Appendix A: Saturation binding assay results for Lab D used to fit the nonlinear one-site binding equation. The mean total added data is the mean observation for each concentration as per the protocol.

Mean Total Added (Molar)	Specific Bound (Molar)
D1-10/25/05	D1-10/25/05
2.13E-10	5.52E-12
2.13E-10	6.88E-12
2.13E-10	6.30E-12
4.48E-10	1.43E-11
4.48E-10	1.56E-11
4.48E-10	1.40E-11
6.06E-10	1.81E-11
6.06E-10	1.86E-11
6.06E-10	1.92E-11
8.69E-10	2.35E-11
8.69E-10	2.55E-11
8.69E-10	2.29E-11
1.30E-09	2.75E-11
1.30E-09	2.59E-11
1.30E-09	2.61E-11
2.13E-09	4.07E-11
2.13E-09	3.91E-11
2.13E-09	3.78E-11
4.40E-09	6.51E-11
4.40E-09	5.76E-11
4.40E-09	5.72E-11
8.87E-09	1.25E-10
8.87E-09	8.38E-11
8.87E-09	9.58E-11